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SKIN TEMPERATURE OF AUSTRALIAN ABORIGINALS UNDER VARYING ATMOSPHERIC CONDITIONS[1]

by

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(Submitted for publication 2nd December, 1937.)

The results of extensive investigations into the heat production of the Australian Aboriginal, originally begun in close collaboration with Dr. F. G. Benedict, of Boston, have been reported from this laboratory by Hicks, Matters and Mitchell (1931), Hicks and Matters (1933), Hicks, Moore and Eldridge (1934).

The main conclusions from the foregoing work which led to the investigations here reported, were that the Aboriginal utilized oxygen at much the same rate as Europeans in conditions comparable with those under which the basal metabolism is generally estimated. The usual methods of computing surface area were found to apply, and apart from a more rapid and extensive "specific dynamic response" to food ingestion, the outstanding impression gained from oxygen uptake measurements in the cold, was that the Aboriginal conserved heat more effectively than the clothed white subject in comparable circumstances, and drew less readily upon his sources of heat production.

In order to test this theory it was decided to make estimations of their skin temperature and of blood flow in the radial artery at the wrist using the Sahli-Jaquet Volumbolograph. The work has extended over five years, during which three expeditions have been made to the Ernabella waterhole in the Musgrave Ranges at approximately lat. 26° S., long. 131° E. In each case the August vacation was utilized for the work, and at this time of the year there is a diurnal swing of temperature from as low as — 4° C. to 37° C.

The native subjects were from the Petermann Ranges to the east and were mostly of the Witjakapandja tribe. Although now well accustomed to contact with whites they still live their normal nomadic existence and are still unclothed. The members of these tribes wear no clothing of any sort, and so are probably the closest approximation to the ideal subject for skin temperature study as outlined by Benedict (1925).

[1] This investigation was carried out with the aid of a grant by the National Research Council from the Rockefeller Anthropological Research Fund.

EXPERIMENTAL DETAILS.

Experimental Conditions. On the first two expeditions observations were made in the early morning before the natives had stirred from their sleeping places and again at mid-day when the subject was exposed to the sun.

The conditions in the early morning camps are illustrated in Fig 1a which is a photograph of one such working camp. The natives sleep side by side on the ground with only a low windbreak of branches or grass about 18 inches high. Between the natives are small camp-fires situated about 2 feet from the body in line with the pelvis. We examined the subjects entirely under their own conditions, no alterations being made except when the subject indicated to his companions that a fire was too hot or required stoking.

Fig. 1a. Photograph of a typical camping place. 1933 expedition. The wind break of brushwood with spears thrown upon it is well shown, and the white patches, some marked with arrows are the remains of fires, between which the aboriginals sleep. The fire at the foot of the subject is exceptional.

At mid-day the subjects were lying fully exposed to the sun and as a rule still protected by the wind-breaks. Usually there were no fires sufficiently large to affect the results.

We attempted to define the atmospheric conditions by the use of ordinary thermometers, wet and dry bulb thermometers, wet and dry kata-thermometer and black bulb thermometer. Even apart from the difficulties of measuring such factors in still air the variable and irregular conditions were such as to make the task hopeless.

On the third expedition an attempt was made to overcome these difficulties by examining the subjects lying in a tent. In this way it was hoped to make the conditions sufficiently uniform to measure. Even then there is considerable difficulty in obtaining a satisfactory estimate of the atmospheric cooling power. These experiments are discussed in more detail in a subsequent paper

(Goldby, Hicks, O'Connor and Sinclair, 1938). Essentially naked white and black subjects were exposed in tents to identical changes in temperature as the atmosphere cooled between 3 p.m. and 7 p.m.

Measurement of Skin Temperature. On the first expedition, owing to rough transport, the thermoelectric measuring apparatus was destroyed, and recourse was made to the use of special flat bulb thin-walled thermometers used by Sir Charles Martin in his own investigations upon skin temperature. These were preheated in the hand of the observer and the lowest temperature attained after application to the skin of the subject, was taken as the skin temperature at that point.

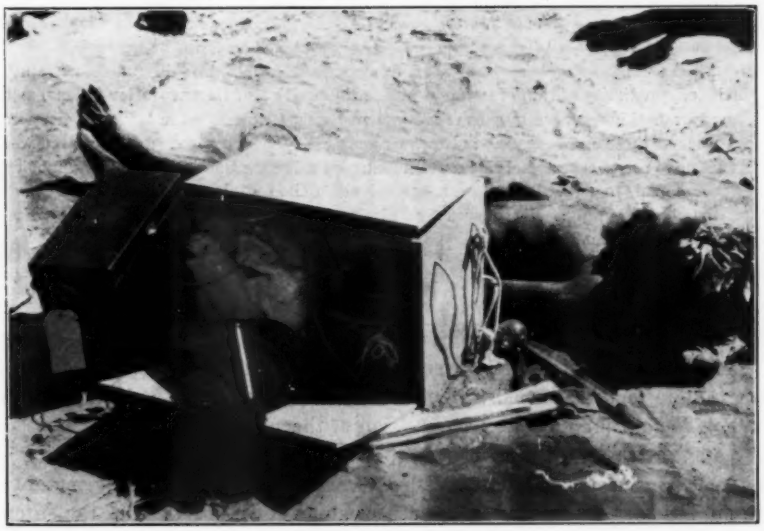

Fig. 1b. Portable thermo-electric apparatus for measuring skin temperature in the field. Cambridge Instrument Coy. electric thermostat for constant temperature junction wrapped in cotton wool with suspension millivoltmeter in same box. Thermometers for air and black velvet and sun temperatures. Thermometer in subject's mouth.

Criticisms of this method have been reviewed by Benedict (1925), Cobet (1926) and by Büttner (1935), but as there was no alternative, and since the relative results under hot and cold conditions will be largely unaffected, we report them here. Moreover, for purposes of comparison with subsequent results obtained by the use of the thermocouple, a correction can be added which enables justifiable corrections to be made. According to Büttner this correction amounts to + 1·5 — 2°C. and is due to (1) shielding of the skin from contact with the colder air and (2) contact pressure by the thermometer bulb, causing closer approximation with the warmer underlying layers of the cutis.

Using Büttner's thermocouple, these factors are obviated. The couple itself consists of of Krupp V_2 A Steel and Constantan of 0·3 mm. and 0·2 mm. thickness respectively, welded together and brought to a point of about 0·1 mm². The wires are formed into a spiral of 2·3 cm. diameter and 1½ turns inside an open ebonite holder which rests upon the skin by 3 small ebonite feet. When lightly applied to the skin it can be demonstrated that whatever the shape of the

surface, the couple does not indent the same sufficiently to cause significant variations in galvanometer readings. Using a Cambridge Instrument Co. suspension milli-voltmeter, readings could be taken in 10 seconds and with a Siemens Schuckert meter in 6 seconds. Benedict, Koropatchinsky and Finn (1928) contend, that using a Benedict copper constantan couple set in an ebonite holder with cotton wool backing, a movement of the couple by ½ inch can give rise to differences of temperature as great as 1°C. Using Büttner's method we have not found significant differences of this kind in the field, and suggest that the high conductivity of the copper, together with the disturbance of "local climate" by the holder may be responsible for these discrepancies. We found that Benedict's thermo-element used in conjunction with our milli-voltmeters required too much time for equilibrium to be established for its use in the field to be practicable.

On all occasions Benedict's (1925) skin topography was made the basis of temperature measurements, so that comparisons with the results of his Institute can be made. On the second expedition the thermocouple leads were not long enough to enable the skin and feet temperatures to be included, proximity of camp fires determining the position of the suspension galvanometer.

The average skin temperatures for the torso on the first expedition in lower air temperatures were the same as those on the second and warmer occasion. The discrepancy disappeared when the correction due to use of thermometers is subtracted. By using the Sahli-Jaquet sphygmobolograph, attempts were made to estimate changes in the volume of the radial artery at the wrist as an index of cutaneous blood supply. For purposes of clarity these latter results are reviewed in a later paper (Hicks and O'Connor, 1938).

RESULTS.

Measurement of Atmospheric Conditions.

Certain characteristics of the conditions in the camps can be defined. Fig. 2 shows the air temperature in a native's "bed" taken about 3 inches above the ground. The outline indicates the position of the subject just before the readings were made. In general it may be said that the air temperature in the bed is 5–10° C. higher than the outside temperature. Certain parts of the body, notably the head and feet, are exposed to temperatures almost as low as that of the outer air. The observations on which Fig. 2 is based were made on a still morning. It was obvious both from a study of the reactions of the natives and from thermometer readings that wind is a very disturbing factor. Windy conditions with air temperature of 5° C. are apparently far more distressing to the natives than are still frosty nights when the air temperature is as low as — 4° C. Radiant heat from the fires was also a factor. Black bulb thermometer readings of 80° F. were always observed alongside the body where a moderate fire was burning. In a few instances readings over 100° F. were observed.

When the subjects were re-examined at mid-day the air temperature was 30–35° C. and the black bulb thermometer readings of the order of 130° F.

The attempts to measure atmospheric cooling power in tents on the third expedition gave results of the type illustrated in Fig. 3a, b, which are two typical examples. The air temperature fell steadily during the progress of the experiments, and at the same time the humidity rose as evening approached. It is interesting to observe that over this range the wet katathermometer actually registered a decrease in cooling power, probably to be attributed to the change in

humidity. The dry kata-thermometer did cool faster as the atmosphere became cooler, but expressed in the usual cooling power units the change was very small. It was apparent that of the characteristics measured, air temperature alone showed the best correlation with skin temperature and with the subjective sensations of the individual, a result which is in agreement with the conclusions of Kisskalt (1909) and Bedford (1935).

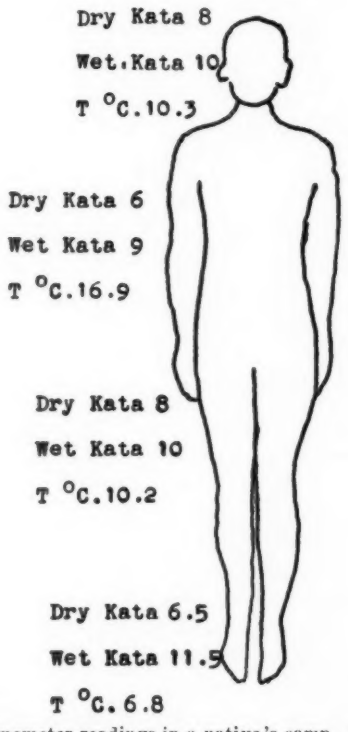

Fig. 2. Dry bulb thermometer readings in a native's camp. The subject had been lying in the position indicated by the outline and was asked to leave his bed. The air temperature was then measured, the results being as shown.

Temperature and Kata values taken at four points along the mid line of a vacated sleeping place between two fires. External air T°C. 5·5, Dry Kata 5·5, Wet Kata 10·0.

Average Skin Temperature.

The average of the skin temperature of all of the areas examined showed considerable differences between the morning (cold) and afternoon (hot) estimations. The actual means were 33·1° C. in the afternoon and 28·2° C. in the morning. This change bears a general relationship to the atmospheric temperature as is

illustrated in Fig. 7, but there obviously can be no close correlation under these variable conditions.

In the controlled cooling experiments a steady fall in skin temperature also occurred, the lowest value reached being of the order of 28–29° C. (see Fig. 3). It is of interest to observe that the same changes in skin temperature occurred in white and native subjects. No difference between the two races could be observed in this respect. This point is illustrated in Fig. 4 in which the skin temperature

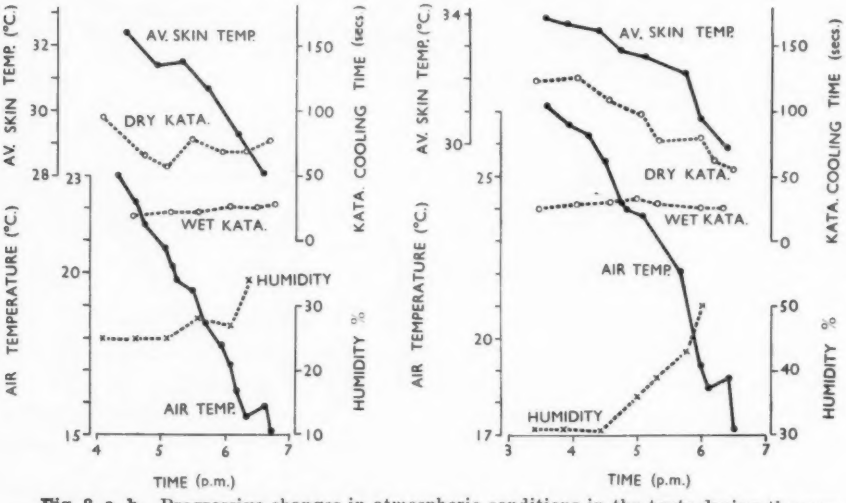

Fig. 3, a, b. Progressive changes in atmospheric conditions in the tents during the controlled cooling experiments. Skin temperature, humidity, air temperature, and the cooling times of wet and dry katathermometer are shown plotted against time. These are two typical examples.

of the native is plotted against the corresponding skin temperature of the white subject. As will be seen a close approximation to a straight line relationship is obtained and the slope of this line is approximately 0·9, showing that as the conditions became cooler the skin temperature of whites and blacks fell in unison. Reference to the examples illustrated will show that there was often 1° C. difference between the absolute values for the two subjects, which is probably to be attributed to the amount of body fat in the different subjects. Although our impression is that this explanation is correct we did not have sufficient subjects to examine this point in detail.

Skin Temperatures of Different Areas.

In considering the regional differences of skin temperature of the first two expeditions the difficulty is immediately experienced that there is no satisfactory

index of the temperature to which the part is exposed. Fig. 5, a, b, c, d, show the skin temperature of the forehead, thorax, abdomen and feet plotted against the average skin temperature of the subject. As will be explained in the discussion of this paper the best index that we have of the atmospheric cooling power is the average skin temperature, so that this figure is used as an index of the cooling power of the atmosphere. It will be seen from the figures that in each case an approximation to a straight line relationship is found, the scatter of the individual

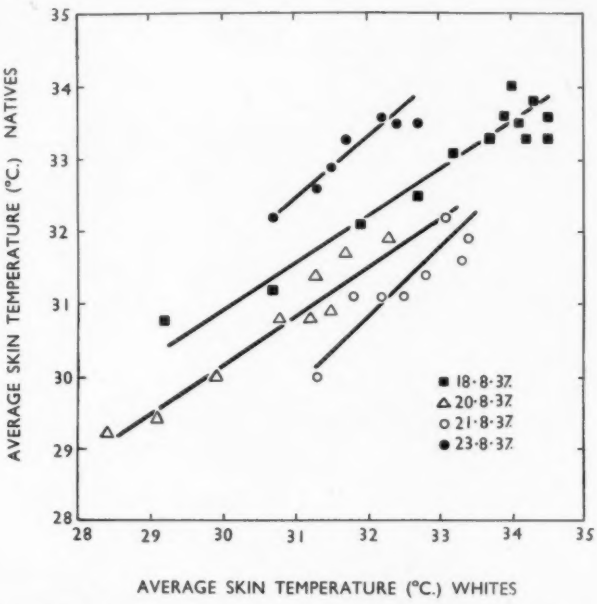

Fig. 4. Average skin temperatures of whites and blacks under corresponding environmental conditions. The skin temperatures of the whites are plotted as abscissae and of natives as ordinates. The points always fall on a straight line the slope of which varies between 0·7 and 1·1.

points being surprisingly small considering the extreme variability of the environmental conditions. The slope of the line in each instance is different. The skin temperature of thorax and abdomen apparently fell in close agreement with the average skin temperature—i.e. the slope of the curve is 1. In the case of the forehead the slope is rather steeper—i.e. the forehead temperature fell more than the average of the whole body in the cold conditions. Likewise the temperature of the the feet fell to values far below that of the rest of the body and showed a still steeper gradient. Considerable care must be exercised in examining these results. Reference to Fig. 2 will show that both feet and forehead are exposed to colder

conditions than the rest of the body and in the use of average skin temperature as the guide to atmospheric conditions this is neglected. Consequently the steep gradient of the curves of Fig. 5 for forehead and feet may be due to the colder

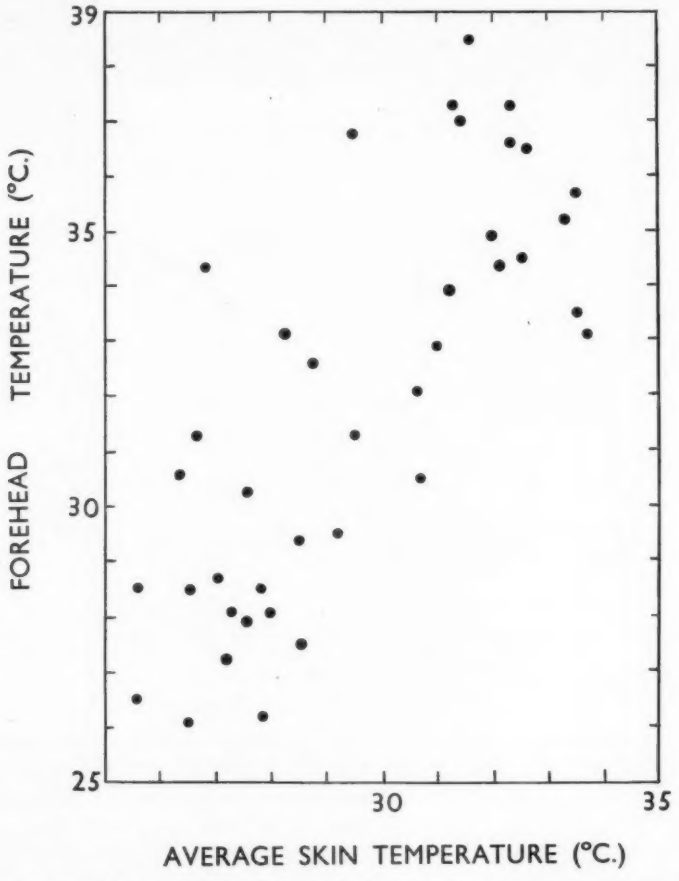

Fig. 5a. The skin temperature of forehead plotted against average skin temperature. The data was that obtained on the natives in their own camps.

conditions to which they were exposed and not represent any fundamental differences.

Fig. 6 shows the behaviour of the skin temperature of various areas in the controlled cooling experiments of the 1937 expedition. Two examples of both native and white subjects are shown. These are typical of the results obtained.

It will be seen that the skin temperatures of the individual areas undergo corresponding falls. The actual figure for each area varies with the subjects. In some cases the temperature of the areas measured (forehead, thorax, abdomen, arms,

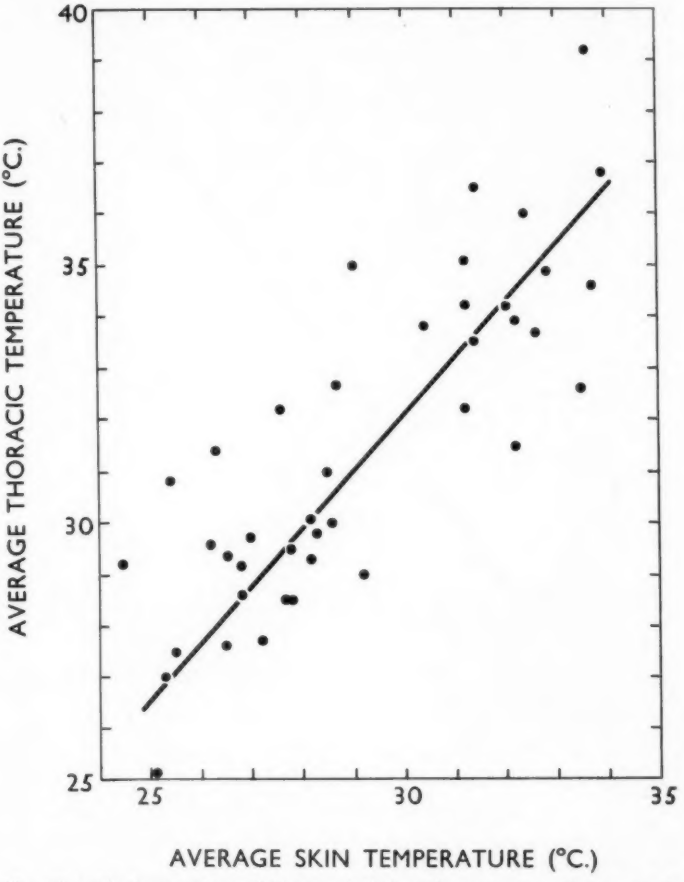

Fig. 5b. The skin temperature of thorax plotted against average skin temperature. The data was that obtained on the natives in their own camps.

thighs) fell close together. In other cases they were widely separated, the temperature of the thighs being in most cases lower than that of the other parts. Although again we have not enough subjects to dogmatize it appeared that the differences between the individual areas is a characteristic of each individual subject and is probably dependent on such body factors as distribution of fat.

Fig. 6 also demonstrates that the natives and whites behaved identically in this respect. Another example of this fact was mentioned in the discussion of average skin temperatures.

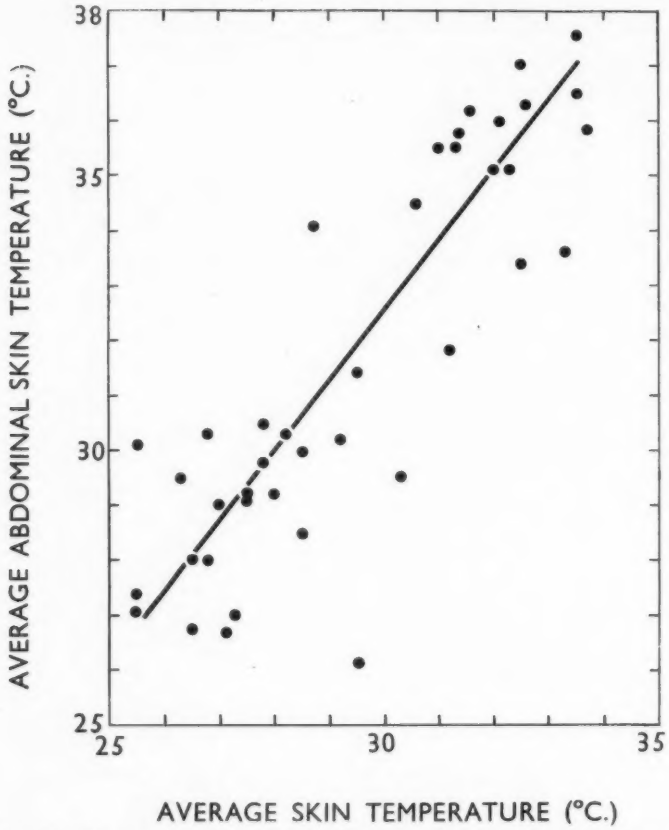

Fig. 5c. The skin temperature of abdomen plotted against average skin temperature. The data was that obtained on the natives in their own camps.

Several authors have paid especial attention to the forehead temperature, claiming that this changes less than does the skin temperature of other parts of the body. Oehler (1904), Reichenbach and Hayman (1907), McLeod and Taylor (1921), Zondek (1922), Bedford (1935). Our results do not substantiate this. In both Figs. 5 and 6 there is no evidence that the skin temperature of the forehead undergoes any smaller changes than do those of the thorax or abdomen. In the

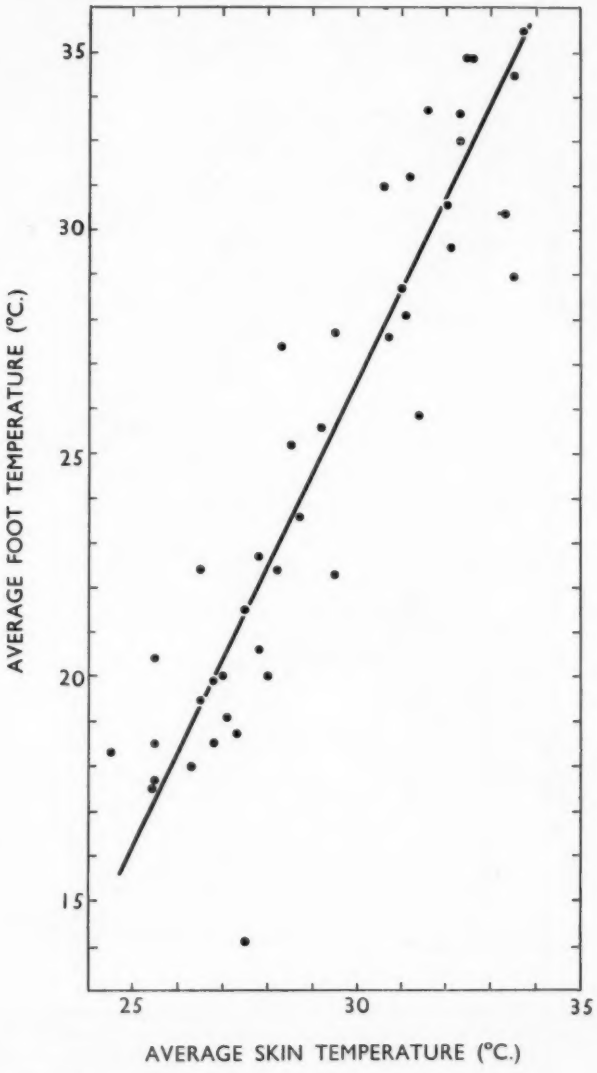

Fig. 5d. The skin temperature of foot plotted against average skin temperature. The data was that obtained on the natives in their own camps.

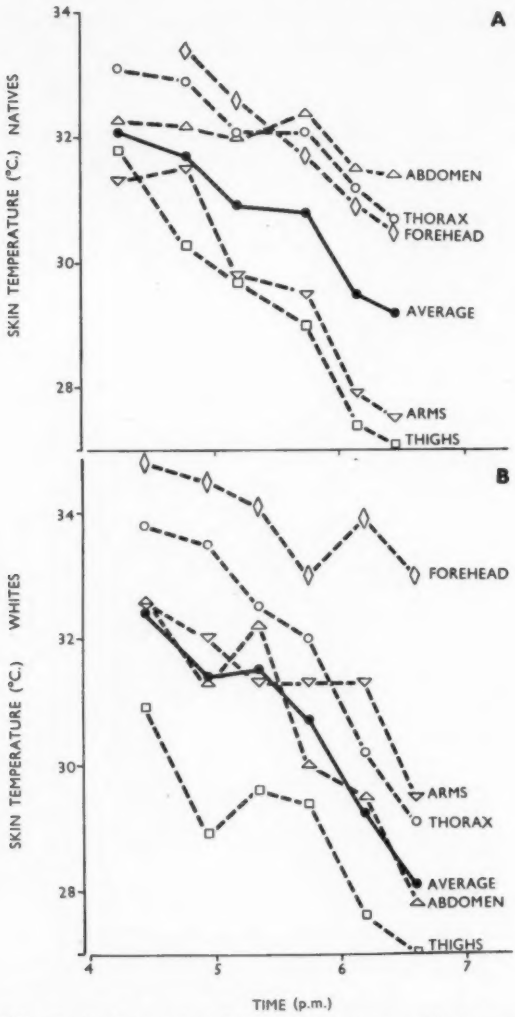

Fig. 6 (a and b). Typical examples of the falls in average skin temperature and the temperature of individual areas in the controlled cooling experiments. Figs (a and b) are those of white and native on the same day.

data obtained from whites illustrated in Fig. 6 it is true that the forehead temperature does not fall as rapidly as that of the other parts, but in all cases our white subjects were flushed from sunburn and in consequence this observation has very doubtful significance.

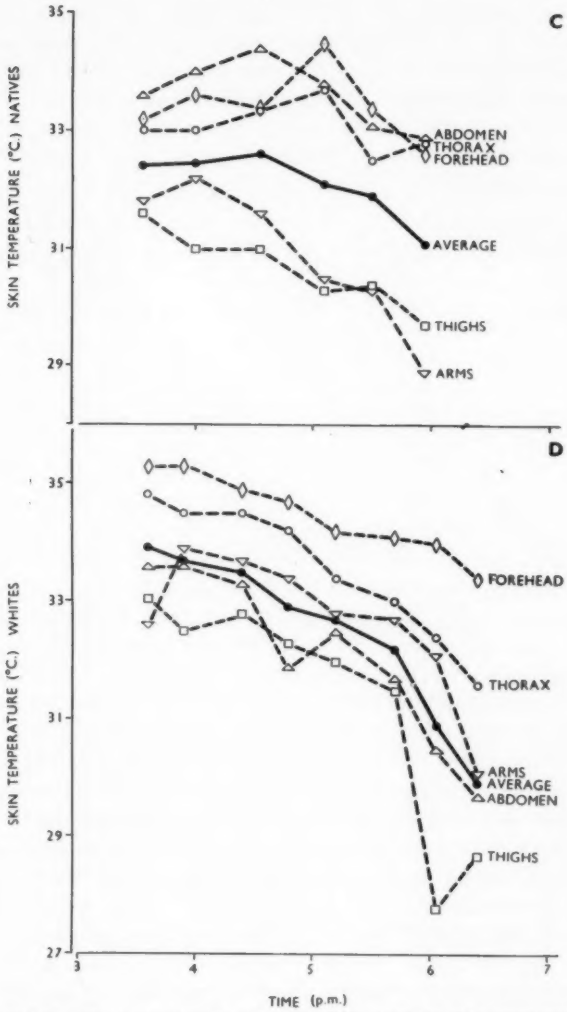

Fig. 6 (c and d). Typical examples of the falls in average skin temperature and the temperature of individual areas in the controlled cooling experiments. Figs. (c and d) are those of white and native on the same day.

Examining the skin temperature readings of the first expedition it was often found that the skin temperature of one side of the body was higher than that of the other. This point was more closely examined on a subsequent expedition when the skin temperature on each side was compared with the black bulb thermometer

reading. Typical results are given in Table 1 where it is apparent that local heating produces an increased skin temperature. This observation does not prove that radiation is the sole or even an important factor in this respect since the heat loss by convection and conduction will equally be minimized by the proximity of a fire.

TABLE 1.

Showing the average skin temperature of the two sides of the body where one side was heated by a fire. The heating effect of the fire is indicated by the black bulb temperature recorded alongside the native. 0 indicates absence of fire.

Right.		Left.	
Black Bulb. Temp. °C.	Skin Temp. °C.	Black Bulb. Temp. °C.	Skin Temp. °C.
0	25·9	29	29·1
0	21·9	28	29·8
28	33·9	0	26·1
0	26·0	8	28·6
0	25·8	8	28·2
0	27·1	25	31·4
32	32·2	12	25·4
28	28·7	0	26·1
0	27·8	48	32·4

DISCUSSION.

From theoretical considerations one would expect the skin temperature to be dependent more on the surrounding environmental conditions than on body responses. We have here observed that the skin temperature of the same subject can change over a range of about 8° C., which is obviously out of all proportion to temperature changes which may occur in the skin as the result of altered cutaneous blood flow. Lewis (1924) in his work on flare used changes in skin temperature as an index of blood flow, but full dilation of skin vessels produced only 1° C. change in the skin temperature. Our results also support this contention. In our controlled cooling experiments the skin of white and black subjects cooled at equivalent rates (Figs. 4, 6). During that range, however, we have evidence that considerable vasoconstriction occurred in the skin of the native and not in that of the white (Goldby, Hicks, O'Connor and Sinclair, 1938), and so it is concluded that the average skin temperature is not significantly altered by peripheral vasoconstriction.

We are, therefore, led to suggest that skin temperature is in our experiments solely an index of environmental conditions and may be used to measure this factor. As an example we may cite the effects of unequal heating of the body in the observations in the camps. A fire warming one side of the body produces corresponding changes in skin temperature. Supporting this contention is the even way in which the skin temperatures of the various parts of the body fell in the controlled cooling experiments. The slightly less rapid fall on the forehead of the white subjects was probably due to the flush resulting from sunburn.

Our contention is strongly supported by some experiments on naked white subjects under artificially controlled conditions by Winslow, Harrington and Gagge (1937). These workers varied both the atmospheric temperature and the radiation to which their naked subjects were exposed, and claim to be able to calculate theoretically an "operative temperature" which measures the sum total of the atmospheric conditions as they influence the heat regulation of the individual. Examining their data we find an extremely close correlation between this

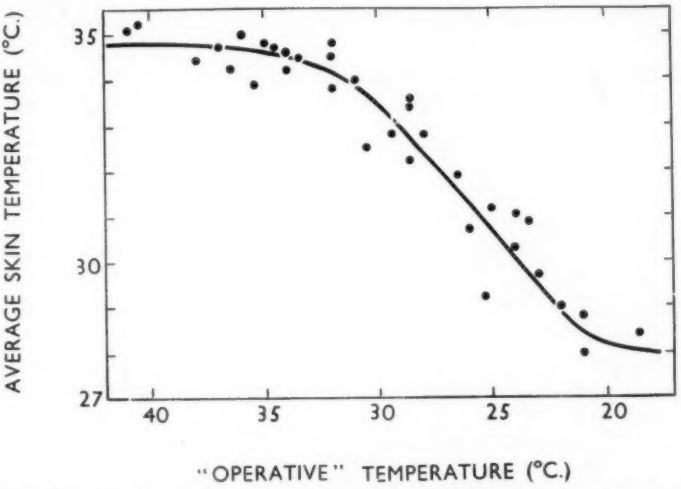

Fig. 7. The skin temperature of naked subjects plotted against calculated effective "operative temperature" of the environment (plotted from the data of Winslow, Harrington and Gagge (1937).

theoretical figure and skin temperature, and by plotting these two factors as has been done in Fig. 7 it is apparent that over the temperature range at which we were working there is practically a straight line relationship. In fact it is a straight line relationship within the order of accuracy to which the "operative temperature" may be calculated. It is apparent that at high "operative temperatures" sweating disturbs the relationship, but this point was never reached in our controlled cooling experiments.

We propose therefore to use the average skin temperature as our index of the atmospheric cooling power. This is subject to certain reservations. The method can be used only in the study of cold conditions owing to the phenomenon of sweating. It is also accurate only to 0·5–1° C.—variations of less than 1° C. are not significant and hence it is almost independent of changes in body temperature or skin circulation. The absolute values for each individual subject are of little importance since the actual skin temperature depends on the build of the subject

in some degree, as was exemplified in the comparison between white and black subjects under identical conditions. Differences of as much as 2° C. in skin temperature may occur between individuals exposed to identical conditions. Changes in skin temperature do, however, represent changes in atmospheric cooling power.

Using this conception we are able to define in some degree the atmospheric conditions to which the native is exposed in his camp. In Fig. 8 the average skin temperatures of the natives are plotted against the air temperature surrounding

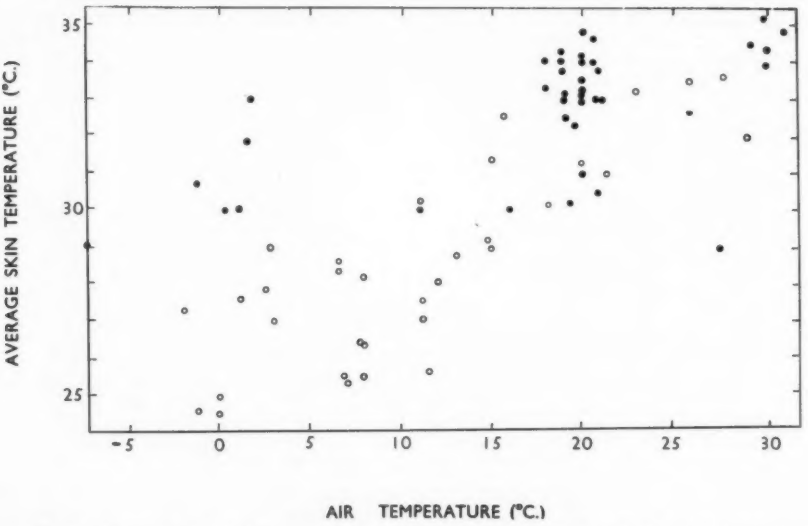

Fig. 8. The average skin temperature of natives (circles) plotted against the air temperature outside the camp. The skin temperature of clothed white subjects (dots) is plotted against the outside air temperature. Data of Benedict (1925) and Benedict and Parmenter (1928).

the camp, the data being that obtained on the first expedition. It must be explained that the camps were entirely natural ones and the natives maintained the fires as they wished. In Fig. 8 the average temperatures of clothed whites are plotted against the surrounding air temperature. The data in this instance have been compounded from those of Benedict and Parmenter (1928). Here also the degree of clothing was that chosen by the subjects in relation to their own comfort. It is obvious that the sleeping native has a skin temperature far below that of the white under similar circumstances, which in our view is merely an indication that the natives' fires and windbreaks are a poor substitute for blankets, and that the natives are in reality exposed to very cold effective climatic conditions.

The establishment of a "normal" skin temperature for aboriginals is obviously impossible if we are to accept the criterion of comfort, as has been done

for clothed subjects by Künkel (1889). The question of comfort in relation to the skin temperature of aboriginals is of interest. We have seen that the natives were able to sleep with skin temperatures as low as 27–28° C., and this must in one sense be an indication of bodily comfort. On the other hand during our controlled cooling experiments natives showed evidence of feeling cold as soon as the white subjects. We are not able to question these native subjects, but the attitude of the subject indicated that he was feeling cold at skin temperatures of about 32° C. while the whites did not feel unduly cold until skin temperatures of about 31° C. were reached. Correspondingly we observed the appearance of gooseflesh on the natives' skin at this stage, and native onlookers would leave the door of the tent and sit around a camp fire. In accordance with this we have often observed a native on waking build up his fire and sit very close to it. In this case part of his body was above the level of the wind-break, but there appears to be no doubt that the native sleeps under conditions which make him feel unpleasantly cold when awake. It is, however, interesting that natives feel the subjective sensations of cold at much the same skin temperatures as our white subjects and at much the same skin temperatures as those observed by Künkel (1889), Rübner (1893), Kisskalt (1907), Cobet, Kotschau and Roelofs (1924), Boyé (1901), on naked or clothed whites.

The study of regional differences in temperature reveals that our work does not indicate any fundamental differences between the skin temperature of the forehead and of other parts of the body. In the natives, forehead temperature behaves in just the same way as that of other parts. It appears that the actual value of the skin temperature at any site depends on the mass of vascular material underlying it and the extent of its insulation by fat. Thus abdominal, thoracic and forehead temperatures, were in our thin subjects all higher than those of the thighs or feet. The only circumstances where forehead temperature behaves differently is in the white subjects where the forehead was flushed from sunburn.

Study of the temperatures of individual areas reveals one possible factor in the natives' ability to sleep under cold conditions. The fires obviously produce areas in which the skin is hot and sufficient radiant heat may be absorbed to maintain equilibrium between heat loss and gain. In this connection there is the observation of Martin (1900) that the black skin of negro races absorbs 30 p.c. more radiant energy than does white skin. The magnitude and importance of this factor in the heat regulation of the native we are unable to assess. Likewise the warm conditions are composed partly of the effects of the radiant energy of the sun. We, however, are not concerned here with the nature of the natives' response to hot climatic conditions.

No attempt has been made to discuss here the nature of the regulatory processes by which the native responds under cold conditions. They will be discussed in detail in the final paper of this series (Goldby, Hicks, O'Connor and Sinclair, 1938).

SUMMARY.

1. Measurements of skin temperatures of the naked Central Australian aboriginal have been made on three expeditions under cold, hot and artificially controlled conditions.

2. The skin temperatures of the natives asleep by their camp fires, were found to be 5° C. below those reported for white subjects in bed, or clothed under similar atmospheric conditions.

3. At a local air temperature of 10°–15° C. under these conditions, the average skin temperature was 27° C., and at 20°–22° C. the average temperature was 31·5°–33·0°C.

4. When the naked black and white subjects were cooled under identical atmospheric conditions their skin temperatures fell to the same extent.

5. Average skin temperature appears to depend mainly upon atmospheric temperature and is thus the most satisfactory index of "cooling power".

6. Optimum temperature and regional difference in skin temperature are discussed.

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THE EFFECT OF CHANGES OF ENVIRONMENTAL TEMPERATURE ON THE SKIN CIRCULATION OF THE NAKED AUSTRALIAN ABORIGINAL, AS MEASURED BY THE SAHLI-JAQUET VOLUMEBOLOGRAPH[1]

by

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(Submitted for publication 2nd December, 1937.)

INTRODUCTION.

In continuation of the work on the Australian aboriginal already published for this laboratory (Hicks, Matters & Mitchell, 1931; Hicks and Matters, 1933; Hicks, Moore and Eldridge, 1934; Hicks and O'Connor, 1938) an attempt was made to estimate the blood flow in the hand by means of the Sahli volume-bolograph. This work was carried out concurrently with that of the previous and subsequent papers of the series (Hicks and O'Connor, 1938; Goldby, Hicks, O'Connor and Sinclair, 1938); but since the results obtained require special discussion to establish their significance and seem also to be of importance in relation to the subject of bolometry itself they are discussed separately in this communication.

EXPERIMENTAL METHODS.

Experimental Conditions. These observations were made at Ernabella, Musgrave Ranges, South Australia, in 1933, 1936 and 1937, and were made at the same time as the skin temperature measurements of the previous paper (Hicks and O'Connor, 1938). In that paper the experimental conditions are discussed in detail. On the first two expeditions the native subjects were examined in the early morning when the air temperature was only a few degrees above freezing point. They were lying close to small fibres shielded by a low windbreak as illustrated in the previous paper. The conditions were irregular but corresponded to a temperature of 10–15°C. in still air (Hicks and O'Connor, 1938). At mid-day the same subjects were examined exposed to an air temperature of about 30°C. and direct heating from the sun. In all cases the subjects were completely naked.

During the third expedition (1937) we endeavoured to obtain more even and measurable conditions by examining the subject lying in a tent. In this instance observations were begun

[1] The expenses of these expeditions were defrayed by grants through the Australian National Research Council from the Rockefeller Anthropological Research Fund.

about 3 p.m. and continued until the temperature had fallen sufficiently to cause discomfort. These results showed a better correlation between skin temperature and pulse volume but as stated in the previous paper we were unable to obtain a satisfactory measure of the atmospheric conditions. A comparison of the reactions of the natives with those of whites under these conditions will be the subject of a further communication. (Goldby, Hicks, O'Connor and Sinclair, 1938.)

The Volume Bolograph (Sahli, 1922, 1928). The instrument used was the Jaquet modification of the Sahli volume-bolograph.[2]

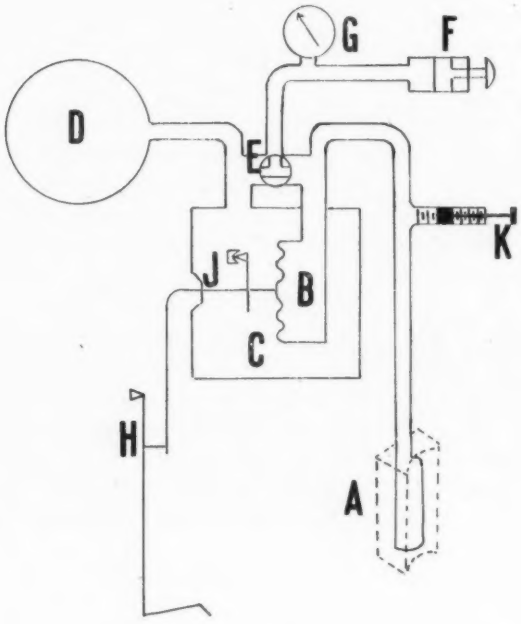


Fig. 1.

A special pelotte is applied to the radial artery. It consists of a small rubber bag, 5 cm. long, bridging a channel cut in a solid rubber block. When this pelotte is tightly strapped to the wrist a tunnel is formed between the rubber block and the lower end of the radius bone. Within this tunnel lies the radial artery and the rubber bag. The pelotte bag, A (Fig. 1) is connected by pressure tubing to a light elastic metal capsule, B, within the instrument. This capsule is enclosed in a box, C, from which a tube leads to an air reservoir, D, of 500 ml. capacity. The capsule and the chamber surrounding it are connected by a three-way tap, E, to an airpump, F, and pressure gauge, G, so that the air pressure can be raised simultaneously in the capsule and the space surrounding it. When a reading is to be taken the tap is turned so that there is no communication between the capsule and the space surrounding it. Variations in pressure as the

[2] Specially made for the purpose by Jaquet et Fils. Bâle.

result of the pulse are thus rendered negligible by the size of the air reservoir and the movement of the membrane records changes in volume of the pelotte at a constant pressure. A light lever system, H, leads from the capsule through an air-tight junction, J, in the outer chamber to enable the movements of the capsule to be recorded on blackened paper. At each pulsation the machine thus records the changes in the size of the radial artery, when a constant external pressure is applied. At each estimation the lever excursion is calibrated in terms of volume by the injection of 0·1 ml. of air into the pelotte via a side tube to which a graduated syringe, K, is connected for the purpose.

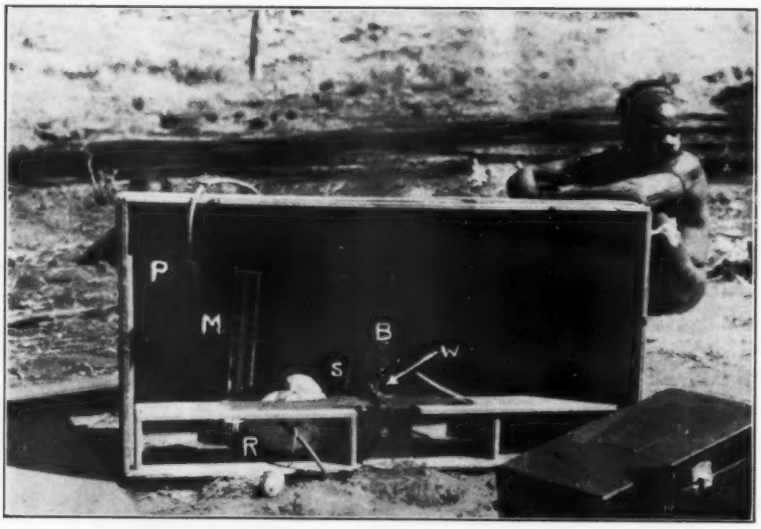
Automatic photograph showing typical field experiment, 1936 expedition. The aboriginal is lying with feet towards the wind-break (the heap of brushwood in right foreground). The arrow indicates smouldering camp fire, on the other side of which, and facing in the reverse direction, he would lie at night. On his chest is the Büttner thermocouple with leads to the thermostat and meter in the case at his left shoulder. In left foreground is the volume-bolograph with pressure tubing leading to the right wrist of the subject.

In making an estimation the pressure in the instrument is set at 67, 70, 80, etc. mm. Hg. and it is seen that the size of the recorded pulse increases to a maximum and decreases again as the pressure in the system is raised. The presure at which the maximum amplitude is obtained is termed by Sahli the "optimum pressure" and the size of the pulse, estimated by comparison with the calibrating injection of 0·1 ml. is here termed the "pulse amplitude". This latter term is used in preference to Sahli's term "pulse volume" since it appears that under these conditions the instrument does not directly measure blood flow.

In mounting the instrument for field use adequate shielding from the wind was essential and the instrument was mounted in a large box, one side of which could be removed to allow access. Even so it was found that rapid changes occurred in the temperature of the air reservoir and this was therefore insulated with cotton wool.

A minor modification was introduced into the technique of using the instrument. Instead, as Sahli recommends, of estimating the optimum pressure by sight and calibrating by injections of 0·1 ml. of air at that pressure a calibrating injection was made at each pelotte pressure as it was applied. This was found more convenient because of the difficulty often experienced in selecting the optimum pressure at sight.

Estimates were made in duplicate or triplicate with a complete re-application of the pelotte at each reading. The accuracy attained was such that individual readings differed from the mean by less than 0·01 ml.



Sahli-Jaquet volume-bolograph mounted on clockwork drive for blackened paper, in box to give shelter from wind. P: Pelotte, M: Manometer, R: Air reservoir insulated in cotton wool, S: Calibrating syringe, B: Bolometer chamber, W: Writing point.

RESULTS.

Effect of General Environment on Pulse Amplitude.

Table 1 presents the results obtained on the 1933 expedition. The figures in column A were obtained in the early morning before the natives had moved from their sleeping positions. As pointed out previously (Hicks and O'Connor, 1938) the wet windy weather encountered at this time probably rendered the natives' fires and windbreaks relatively ineffective, and it is probable that the subjects were exposed to conditions equivalent to temperatures of 5–10° C. in still air. In column B are given the figures obtained with the subject lying in the sun and protected from wind. There was a much smaller pulse amplitude in the cold—of the order of 50 p.c. of that observed when the same subject was warm later in the day.

Effect of Local Heating on Pulse Amplitude.

In the camps in the mornings one side of the native was often heated by a warm fire while the other was exposed directly to the cold. Corresponding to these conditions we found gross differences in the skin temperatures of the two sides of the body (Hicks and O'Connor, 1938). Table 2 shows the pulse amplitudes obtained from the two wrists under these conditions, and it is seen that in general the pulse amplitude is greater on the hot side. The results do not warrant more than this general statement. The conditions changed so rapidly that the skin temperature either of the hand or of that side of the body cannot be used as more than an indication of the extent of the local heating. The posture of the hand before and during skin temperature or pulse amplitude readings was an uncontrolled but important factor. From the results we are able to state that local heating affected the circulation in that part, but we are not able to say whether the heating of one part of the body affected the circulation in remote parts.

TABLE 1.

Pulse Amplitudes of the Same Subject under Cold and Hot Conditions (1935 Expedition).

Subject.	Pulse Amplitude.	
	Cold.	Warm.
140	0·04	0·12
44	0·03	0·11
51	0·03	0·14
136	0·08	0·12
139	0·05	0·09
138	0·05	0·10
49	0·07	0·12
17	0·08	0·10
142	0·06	0·11
35	0·06	0·12
29	0·07	0·14
X3	0·08	0·19
134	0·08	0·11
147	0·07	0·10
40	0·06	0·11
137	0·08	0·19
28	0·14	0·18
133	0·08	0·14
4	0·08	0·16
27	0·14	0·20

Pulse Amplitude under Controlled Atmospheric Conditions.

During the 1937 expedition an attempt was made to obtain more even atmospheric conditions by examining the natives lying in a tent. Observations were begun at about 3 p.m. and continued as the atmosphere cooled. A comparison of the reactions of native and white subjects will be made in a subsequent paper. Unfortunately the weather was windy and consequently unsuitable for this work, and, as has been explained in the previous paper, we were unable to find any one

figure which represents the cooling power of the atmosphere. However, if pulse amplitude is plotted against the average skin temperature a surprisingly good correlation is obtained. Fig. 2 shows such a curve for one of our native subjects and is typical of the results obtained with each of the seven subjects that we examined in this way. The points plotted were actually obtained on three different afternoons and consequently show a most satisfactory agreement. It appears that, in native subjects, the pulse amplitude is at its maximum at skin temperatures above 33° C. and at its minimum below 31° C. Between these limits there is a progressive fall in pulse amplitude as the temperature falls.

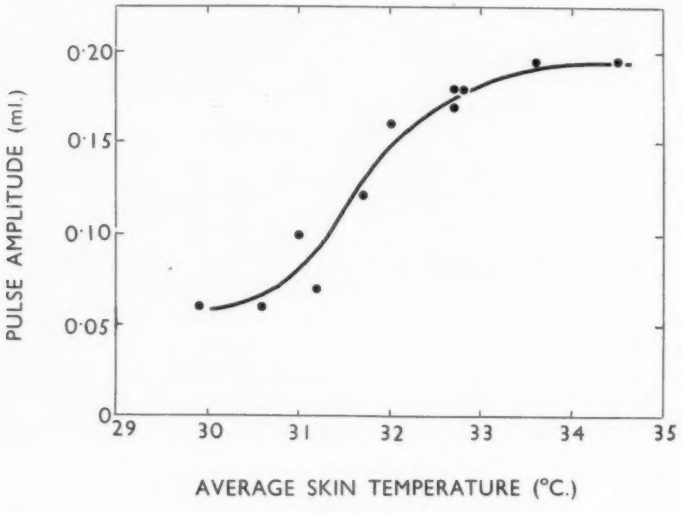


Fig. 2.

An analysis of the skin temperature readings showed that even cooling was obtained and the skin temperature in the arm followed the general average quite closely (Hicks and O'Connor, 1938). Therefore these results do not show whether the pulse amplitude alters in response to local or general changes in temperature.

Changes in Optimum Pressure.

In examining the bolometer tracings it is possible to measure the pulse amplitude at each individual pelotte pressure which is applied. These two factors, plotted against each other, give a curve of characteristic shape. Under the conditions of this work the shape of this curve showed typical changes as the pulse amplitude fell. Fig. 3 illustrates four such curves obtained from one subject. Curves I and II were obtained from the right and left wrists when the subject was

exposed to the sun. The pulse amplitudes were 0·15 and 0·14 ml. respectively and in each case the optimum pressure was 80 mm. Hg. Curve III was obtained in the morning from the right wrist which was warmed by a fire. It is seen that the peaked top of curves I and II is flattened and the optimum pressure is 90 mm. Hg with a pulse amplitude of 0·09 m. Curve IV, from the left wrist which was

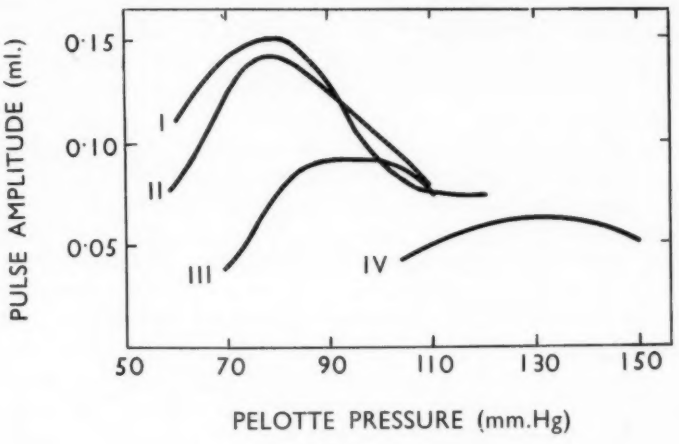


Fig. 3.

extremely cold, shows an extremely flat top, an optimum pressure of 130 mm. Hg. and a pulse amplitude of only 0·06 ml. This series is typical. In all cases, as the pulse amplitude falls in response to cold, the curve becomes flat-topped and the optimum pressure increases.

TABLE 2.

Effect of Local Heating as shown by a Comparison of the Pulse Amplitude on the Two Sides.

Right Arm.			Left Arm.		
Skin Temp. °C.		Pulse Amp. ml.	Skin Temp. °C.		Pulse Amp. ml.
24·8		0·11	29·8	F	0·12
21·9		0·04	28·0	F	0·15
35·0	F	0·11	26·3		0·13
29·6	F	0·13	25·9	F	0·10
24·5		0·09	29·1	F	0·12
29·5		0·11	35·3	F	0·16
35·2	F	0·20	24·7	F	0·18
28·5	F	0·13	26·2	F	0·08
26·9	F	0·15	34·3	F	0·15

F indicates the presence of the fire on that side.
Skin Temp. was taken on the anterior surface of the forearm.

DISCUSSION.

The results clearly show that there is a relationship between the heating of the body and the pulse amplitude. As the skin temperature falls there is a fall in pulse amplitude, and in association with this there is a change in the shape of the curve relating pulse amplitude and pelotte pressure. It is known that cooling, either of the arm itself or of the other arm, causes a diminution of the blood flow through the hand due to vasoconstriction (Stewart, 1912 a; 1912 b, 1915). Apart from such physiological considerations, the differences observed in readings taken almost simultaneously from the two arms demonstrate that these changes in bolograph readings are of peripheral and not of cardiac origin. It has been shown in an experimental analysis of the bolograph that obstruction to the flow distal to the pelotte produces no change in the pulse amplitude (Hediger, 1922). The same experiment may be performed by compressing the radial artery peripheral to the pelotte and again the reading is not affected. It appears therefore that arteriolar constriction does not constitute of itself the explanation of the observed changes.

From an analysis of the conditions in the length of radial artery under the pelotte it appears that the volume change at each pulse beat consists of two portions. Under suitable pelotte pressure the artery is collapsed during diastole. During systole the pressure inside the artery rises sufficiently to fully distend it and hence there is a volume change depending on the calibre of the radial artery if exposed to no pressure (internal or external). If the pressure in the artery rises still further the artery is expanded against the elasticity of its walls and a further movement of the bolometer results. Thus the recorded amplitude consists of two parts—a factor dependent on the calibre of the radial artrey and a factor dependent on the elasticity of its walls. Under the conditions of our work, where relatively small changes in cardiac output are to be expected the pulse amplitude will thus depend on the state of contraction of the radial artery itself. Obviously the calibre of the vessel and the elasticity of its wall will change in unison, and so the relative size of the two factors is of no immediate importance. The changes which occur in the curve correlating pulse amplitude and pelotte pressure agree with this explanation. The flat top of the curve and the increased optimum pressure in the contracted artery are probably due to its rigid walls. Greater pressure is needed to deform and collapse the more rigid vessel, and in the extreme case the wall is so thick that the artery cannot be occluded by external pressure. Similar difficulties occur in the measurement of arterial blood pressure in arterio-sclerotic subjects.

This analysis of the meaning of bolometry does not take into consideration the effects of changes in cardiac output which, under the conditions of our work, must be small compared with the observed alterations in pulse amplitude. When used in clinical work the peripheral changes are negligible, and under these conditions the pulse amplitude probably does reflect the cardiac output. Our data can do no

more than emphasize the possibility that peripheral changes may interfere with the use of the instrument for estimating cardiac output.

We believe, therefore, that under the conditions of these experiments *the bolograph readings measure the state of contraction of the radial artery.* We do not claim that there is any direct proportionality between the pulse amplitude and the blood flow through the hand. It has been shown that the blood flow through the hand does diminish as the hand cools (Stewart, 1913). We consider that the vasoconstriction in the skin is only part of a general constriction of the whole arterial tree leading to that region and therefore our bolographic measurements on the radial artery indicate the process of vasoconstriction in the skin vessels and consequently the blood flow in the skin of the hand. Despite these difficulties in interpretation the bolometer has advantages over other methods of following changes in the skin circulation of the hand. Both the hand calorimeter (Stewart, 1912) and the hand plethysmograph (Hewlett, 1910) surround the hand by a fluid medium the temperature of which cannot readily be changed to apply a local stimulus. The bolometer, however, leaves the hand exposed to the atmosphere and hence enables local atmospheric conditions to be regulated.

Our results indicate therefore that the walls of the radial artery contract when the subject is exposed to cold, and we believe that this is part of a general vasoconstriction of the arterial tree leading to the skin of the hand. This interpretation is supported by the observation that rapid changes of cooling power which resulted from a cloud obscuring the sun in windy, wet weather caused a rapid rise of as much as 30 mm. Hg in the systolic blood pressure of the naked native. Repetition of such weather has not occurred on subsequent expeditions, so we have not been able to amplify this observation. Thus our results agree with the accepted changes in skin circulation in response to cold, but emphasize the fact that the whole arterial tree and not only the arterioles are involved.

These results could be explained on the theory that the circulation in one hand is dependent on the temperature of the hand itself and that the temperature of other parts of the body is unimportant. Stewart (1912), however, has shown that heating one hand influences the circulation in the other. As has been explained the comparison of the two sides when exposed to unequal heating was complicated by the irregular conditions, and hence we are unable to confirm Stewart's results. Our impression was that extremely small pulse amplitudes were not obtained in the morning unless the whole body was cold, and so we believe that the reflexes affecting the circulation in the skin of the hand can be initiated both from the hand itself and from other parts of the body.

In this discussion skin temperature has been used solely as a measure of local heating or cooling. It was shown in the previous paper that skin temperature depends almost entirely on the local environmental conditions, and in these experiments at least it is but little affected by the circulation in the skin or by the body

temperature. The close correlation between skin temperature and pulse amplitude does not mean that change in skin temperature is of necessity the important stimulating factor.

SUMMARY.

1. The Sahli Volume-bolograph has been used to measure the radial pulse of naked Australian Aboriginals exposed to extreme changes in environment.

2. The pulse amplitude diminished with exposure to cold and increased with exposure to heat. Similar changes resulted if one hand was heated and not the other. Changes in optimum pressure also occurred.

3. It is concluded that these bolograph readings measure the state of tone of the walls of the radial artery and that they are related to vasoconstriction of cutaneous blood vessels of the hand.

4. The nature of the stimulus to alteration in the tone of the peripheral vessels is discussed.

ACKNOWLEDGMENTS.

We wish to acknowledge our indebtedness to Mr. Stan. Ferguson, of Ernabella. His hospitality and co-operation alone made these expeditions possible.

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A COMPARISON OF THE SKIN TEMPERATURE AND SKIN CIRCULATION OF NAKED WHITES AND AUSTRALIAN ABORIGINALS EXPOSED TO SIMILAR ENVIRONMENTAL CHANGES[1]

by

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(Submitted for publication 2nd December, 1937.)

INTRODUCTION.

Previous papers of this series (Hicks and O'Connor, 1938 a, b) have reported the changes which occur in the skin temperature and hand circulation of naked Australian aborigines exposed to extremely cold conditions. As has been discussed in the previous paper (Hicks and O'Connor 1938 a) the environmental conditions were so variable that it was not possible to compare the reactions of the native with those that have previously been reported for white subjects.

In the experiments to be reported here white and black subjects were exposed to identical cooling conditions so that a direct comparison could be made. Certain of the results have been discussed in the previous papers of this series, but it is proposed in this communication to make a detailed comparison of white and native subjects in respect of such characteristics that we have studied.

EXPERIMENTAL CONDITIONS.

The experiments were made at Ernabella, Musgrave Ranges in August, 1937. The black subjects were Myall blacks of the Witjakapandja tribe, still accustomed to living completely naked. Seven black subjects were used. All were males of approximately the same ages as the four white subjects comprising the members of the expedition. The development of body fat was similar and of normal average in all subjects.

Two tents were pitched side by side. In one a white subject, in the other a black lay naked on the ground. The experiment was started about 3 p.m. and continued as the air temperature fell until the subject was unable to withstand the cold any longer. As a rule the work finished about 6.30 p.m. During this period repeated estimates were made of skin temperature of the anterior surface of the body by means of a Büttner thermocouple (Hicks and O'Connor 1938a),

[1] The expenses of this expedition were defrayed by a grant by the National Research Council from the Rockefeller Anthropological Research Fund.

of pulse amplitude by means of the bolometer (see previous papers), pulse rate and mouth temperature while the air temperature, humidity, and cooling power by the wet and dry katathermometer were recorded in each tent. In order to accustom the whites to being unclothed the subject lay naked in the tent for about an hour before the work began.

RESULTS.

Environmental Conditions.

A detailed account of our attempts to measure the environmental conditions has been presented previously (*loc cit.*). It was there concluded that no one figure could be used to represent the cooling power of the atmosphere. The data, however, were sufficient to show that the atmosphere of the two tents followed the same changes and so our main object, the exposure of native and white subjects to the same environmental changes, was achieved. Our data enable us to say that the warmest conditions to which our subjects were exposed were:

Air temperature, 29·8° C.
Humidity, 35 p.c.
Cooling power by dry katathermometer 2 millicalories/cm.^{2}/sec.
Cooling power by wet katathermometer 17 millicalories/cm.^{2}/sec.
Wind velocity, 20 ft. per sec.

The coldest conditions encountered were:

Air temperature, 15° C.
Humidity, 36 p.c.
Cooling power by dry katathermometer 6·6 millicalories/cm.^{2}/sec.
Cooling power by wet katathermometer 18 millicalories/cm.^{2}/sec.
Wind velocity, 20 ft. per sec.

This full range was not covered in any one day. As a rule there was steady fall over about two-thirds of this range during the three hours of the experiment, i.e. the air temperature fell about 10° in this time. In general the fall was a fairly steady one except for about the last half-hour. At this period the sun passed behind a hill and there was a more rapid drop of 3–4° in this half-hour. The fall was, however, never sudden enough to suggest that the rate of temperature change was a major factor affecting our results.

Skin Temperature: Skin temperature was estimated at ten different points —the forehead, the two sides of the chest, the epigastrum and both sides of the abdomen, each forearm and each thigh. Since the subjects were lying on their backs throughout the observations these were taken as representative of the parts exposed to the atmospheric changes.

Comparing the averages of these ten points it was found that there was no significant difference between the figures obtained for whites and blacks. In both cases the average skin temperature fell from the maximum figure of 34°–35° C. to minimums of the order of 28°–29° C. Actually only about two-thirds of this range was covered in any one day, but in every case there was agreement within 1–2° C. between the skin temperatures of native and white at any time. Certainly

no regular difference could be discerned. This has been discussed in detail in a previous paper, whilst a discussion of regional differences in skin temperature has already been presented when it was shown that the responses of whites and natives were identical in this respect (Hicks and O'Connor 1938 a).

Mouth Temperature: In the absence of rectal temperature measurements mouth temperature was recorded. The native subjects invariably showed a fall while the whites only showed a small and irregular change. The average fall in the case of the natives was 0·9° F. and in whites 0·3° F., a difference which is certainly significant. Although extreme care was taken in placing the thermometer

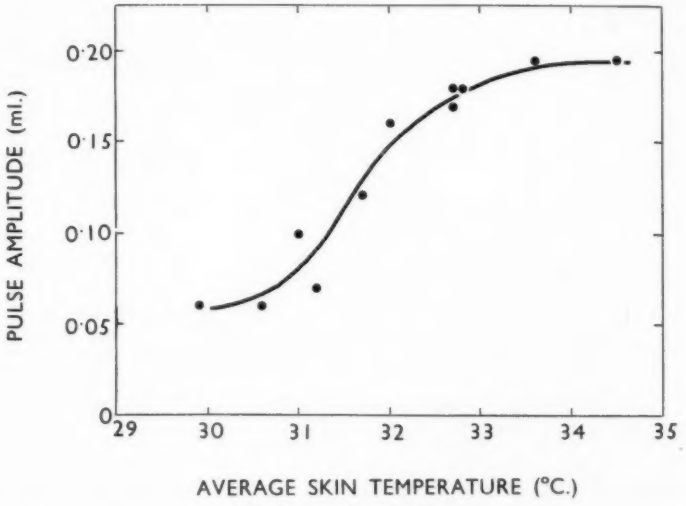

Fig. 1. Black subjects.

under the tongue of the natives it is not possible to be certain that it was maintained in the correct position, and for this reason it cannot be accepted that these differences in mouth temperature represent true differences in body temperature. A possible additional factor will be discussed later.

Pulse Amplitude: It was shown in the previous paper that readings of the pulse amplitude by the Sahli volume-bolograph provide an indication of the state of constriction of the radial artery, and that this artery became constricted in response to cold. This constriction of the radial artery is believed to be part of a general vasoconstriction of the vessels supplying the skin of the hand. Under the conditions of these experiments it was found that a close correlation could be drawn between skin temperature and pulse amplitude. Fig. 1 shows the typical curve obtained from a native, while Fig. 2 shows a similar curve obtained from a

white subject of about the same age. In all cases it was found that the pulse amplitude fell when the skin temperature fell below 33° C. whereas in whites temperatures of 30–31° C. were reached before this change began. This is further illustrated by Figs. 3 (a and b) where all of the bolograph readings obtained on white and black subjects are plotted against skin temperature. In this case the pulse amplitude is expressed as percentage of the maximum attained by that subject, and considering the number of subjects and the different climatic conditions under which the work was carried out a surprisingly close agreement was obtained. The difference between the series is quite evident. At skin temperatures of 32° C. the

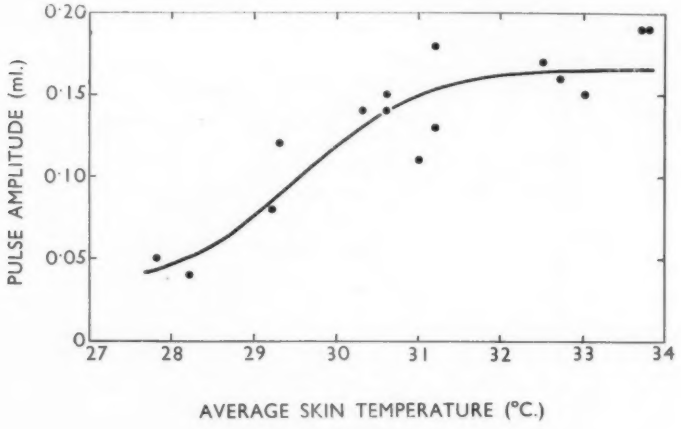

Fig. 2. White subjects.

natives have pulse amplitudes of 70 p.c. and less whereas the white still have full arterial dilation. Similarly at 30° C. the natives show only 30 p.c. of their full pulse amplitudes whereas in the case of the whites values above 50 p.c. are obtained. There is no difference in the nature of the response in the two series. The difference is merely that the threshold skin temperature in the case of the natives is about 32–33° C. whereas in whites it is 30–31° C. The process is therefore more effective in conserving body heat in the case of the habitually naked black than in the clothed white subject.

Subjective Sensations: The whites at skin temperatures above about 33° C. felt pleasantly warm and comfortable. As the temperature fell below this level the subjects felt cool but this was stimulating rather than unpleasant. At skin temperatures of about 31° C. the subjects began to feel definitely cold and would have resumed their clothes if free to do so. Below this level an unpleasant sensation of cold was felt and became more intense. Goose-flesh appeared on the skin at about 30° C. and definite shivering at about 28° C. At these final stages the subject felt miserably cold.

In attempting to estimate the corresponding sensations in the native we are entirely dependent on our observations of the subjects. The interpreters available are unable to do more than put leading questions, and our experience has convinced

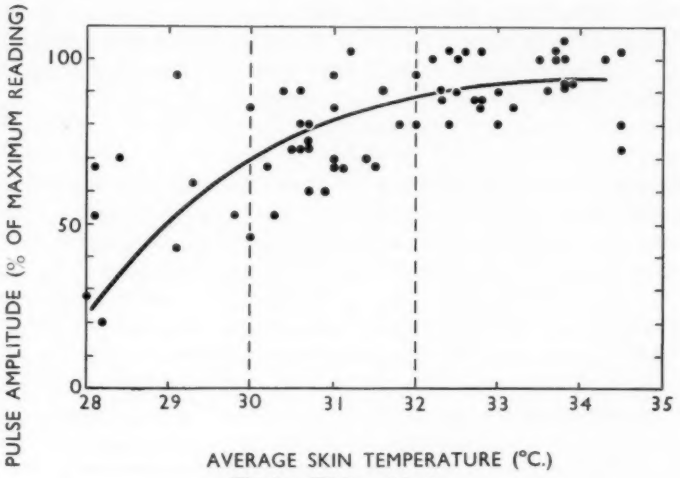

Fig. 3a. White subjects.

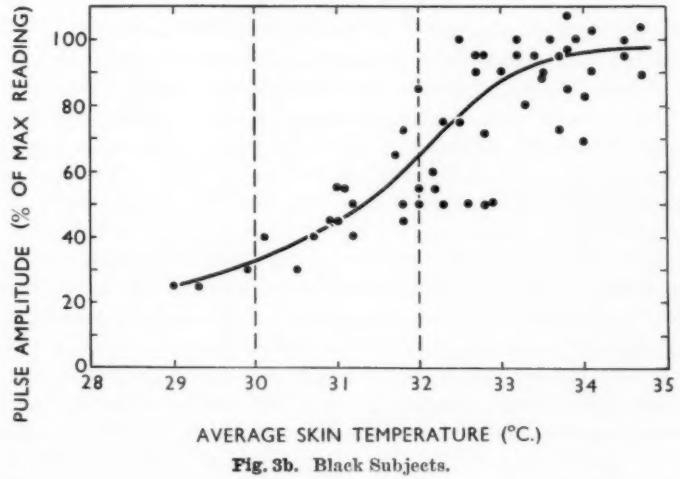

Fig. 3b. Black Subjects.

us that the answers so obtained are totally unreliable. Our observations, however, left no doubt that the natives felt unpleasantly cold before the whites did so. Gooseflesh appeared and the natives looked miserable and hunched themselves up

when the skin temperatures were still approximately 32° C., whilst other natives who gathered at the tent door during the experiments would go away and sit around a camp fire at this stage.

It appeared that in general the gooseflesh and the subjective sensations of cold occurred at the same time as the peripheral vasoconstriction, a fact which may be of importance in relation to the nature of the stimulus which causes this latter response. The relationship of skin temperature to bodily comfort has been discussed previously (Hicks and O'Connor 1938 a).

DISCUSSION.

The results show certain clear-cut distinctions between native and white subjects exposed to identical cooling conditions. As atmospheric conditions become cooler the native limits his skin circulation long before the white is able to do so. The native shows a definite fall in mouth temperature which does not appear in whites and definitely feels the cold earlier. On the other hand the skin temperatures of the whites and blacks undergo the same changes. This fact has already been discussed and the conclusion reached that, over the range of atmospheric conditions that we are considering, skin temperature depends entirely on the atmospheric conditions and can be used as a measure of changes in atmospheric environment.

Using skin temperature as a measure of atmospheric cooling power we have shown that the bolograph readings diminish in the natives almost as soon as air conditions become such that heat loss of any magnitude is occurring. The whites do not respond in this way until much lower temperatures are reached. The significance of bolometry under these conditions has been discussed in the previous paper (Hicks and O'Connor 1938 b) and the conclusion reached that what we have measured is the state of constriction of the radial artery. This, we believe, reflects the state of constriction of the arterioles of the skin of the hand and so indirectly provides a measure of the skin circulation in that locality. Thus we may reasonably generalize that a far greater cooling power of the atmosphere is required to stimulate reduction of the skin circulation of the normally clothed white than is the case in the naked aboriginal exposed to the same environment.

In association with this active vasoconstriction in the skin vessels we find that the mouth temperature of the aboriginal falls. We have indicated difficulties in the obtaining of mouth temperatures of the natives, but in any case the mouth temperature must also be affected by the blood flow in the oral mucous membranes. It is possible therefore that the falling mouth temperature of the natives is due to active vasoconstriction in the mouth. Hill and Muecke (1913) and Spiesmann (1936) have shown that cooling of part or whole of the body produces such a change in the mucous membranes, and this question appears worthy of further study in these habitually naked subjects, amongst whom respiratory diseases are rare.

REVIEW OF HEAT REGULATION OF THE NATIVE UNDER COLD CONDITIONS.

Certain facts have now been established with regard to the way in which the naked native is able to maintain his body temperature under extremely cold conditions. Quite regularly the native is exposed at night to environments which despite his fires and windbreaks are far colder than those to which a clothed European is ever exposed. Both from direct measurement and from the study of skin temperature we are led to believe that the average conditions in the native camps in early morning are equivalent to air-temperatures of 10–15° C.—conditions which from our personal experience in the controlled cooling experiments would be unbearably cold to a naked white, and certainly would render sleep impossible.

We cannot state as yet the effectiveness of the regulatory processes in the natives. In all cases we have observed low mouth or axillary temperatures in native subjects when exposed to cold. This, however, is attributable to other causes than a falling body temperature, and until we are able to measure rectal temperature we cannot make any definite statements as to constancy of body temperature under these cold conditions.

The features of the adjustment by the native to meet these circumstances, as far as we have been able to determine them, are as follows: (1) As the result of the use of small fires to which he is able to lie very close, areas of intensely high skin temperature are found on the body of the sleeping native. It is possible that in this way a large amount of radiant heat is absorbed so that the balance can be preserved despite the fact that other parts of the body are so intensely cold. The magnitude of this factor we have not determined, but it appears probable that the conception of a rapid loss of heat from the cold parts, notably the extremities, balanced by heat absorption at hot areas is a correct one.

(2) The native has an extremely active control of his skin circulation, and heat loss from a cold part is minimized by a diminution of the circulation through that part. Moreover it appears that this control is more definitely localized than is the case in the white. We do not wish to imply that the circulation in any one part is independent of the temperature of the rest of the skin. Considerable constriction of one radial artery may however occur with a full circulation in the other. This localized response is obviously a most effective method of heat regulation when considered in relation to the irregular heating of the body which we have described. It is this combination of the maximum limitation of circulation in the cold areas and consequently of heat loss, with a considerable uptake of heat from the areas heated by fires, that we believe to constitute the means by which the native is enabled to maintain his body temperature.

(3) Despite the cold environment the metabolism of the native does not increase. We have not as yet compared the responses of whites and natives under identical conditions in this respect, but there is no doubt that whites under condi-

tions approaching those of the native would show such a change. Hill and Flack (1914), Campbell, Hargood-Ash and Hill (1921) and Martin (1930) have all reported increases of 30 p.c. in the metabolism of whites before shivering occurred. These results were not obtained by Loewy (1890), Johansson (1896) or more recently by Winslow, Harrington and Gagge (1937). Our natives were exposed to conditions far colder than those reached by any of these workers. A study of metabolic responses in whites and natives under identical conditions will be of the greatest interest. Preliminary attempts were made on this last expedition, but were not completed owing to inadequate technical assistance.

In association with this study it was of the utmost interest to observe that the natives experienced the subjective sensations of cold just as keenly as did whites. This was apparent during the controlled cooling experiments, and often we have observed a native wake and sit up in his camp and build up a fire to warm himself. The native may feel cold when awake although his position is unchanged from that in which he has been sleeping. We suggest that the explanation of the apparent anomaly of a native being able to sleep despite subjective sensations of cold lies in the possibility that the wakefulness of a cold white subject is not due to the stimuli arising from the skin but is due to the state of sympathetic tone which results from cold. The increased metabolism is only one manifestation of this increased adrenal activity. In the native, however, no such general response occurs, and in consequence the vagotonic state necessary to sleep is not reversed. The native maintains his body temperature by a local reflex controlling the skin circulation and the general autonomic system is not involved.

This view fits well with the nature of the aboriginal as we know him. The responses of the sympathetic system are all very slow. As examples we may quote their stoicism during initiation and sacred ceremonies. J. B. Cleland observed the pulse rate of a lad throughout a circumcision operation and found no change.[2] Corresponding to this we have the placid way in which these subjects submitted to these experimental procedures. It is amazing that it is possible to place a mouthpiece in a subject's mouth and carry out a metabolism estimation with no other explanation than can be given by dumb-show. The same subjects show extraordinary ability to lie absolutely motionless for as long as an hour at a time while we carried out our measurements. To complete the picture we find that the blood pressures of the natives are uniformly low compared with that of whites, and pulse rates over 60 are rare, the average being only a little above 50 beats per minute. The observation that the native is slow to raise his metabolism in response to cold is therefore not surprising and reveals an interesting distinction between the subjective sensations and the wakefulness associated with cold.

[2] Personal communication.

SUMMARY.

1. Naked Australian aborigines and unclothed white subjects have been exposed to identical conditions of cooling.

2. The skin temperature falls to an equal extent in the two races.

3. Volume-Bolograph measurements demonstrate that the radial artery of both races constricts in response to cold but that this response occurs with only slight cooling of the natives, whereas intense cold is required to produce a similar response in whites. From this it is concluded that the native has a more effective regulation of heat loss by means of vasoconstriction in the skin in response to cold.

4. The oral temperature of the natives fell during the experiments whereas that of the whites did not. It is suggested that this is due to a reflex constriction of the vessels of the mucous membranes of the mouth rather than to a fall in blood temperature.

5. The processes of heat regulation in the Australian aboriginal under cold conditions are reviewed.

ACKNOWLEDGMENT.

Our thanks are due to Mr. J. B. Love, of the newly-established Mission at Ernabella, for his assistance.

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THE CHOLINESTERASE CONTENT OF BLOOD SERA FROM NORMAL AND MYOPATHIC SUBJECTS

by

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Studies of the rates of hydrolysis and re-synthesis of acetylcholine by normal and myasthenic sera have previously been reported from this laboratory by Hicks and Mackay (1936). In that work the Straub frog's heart preparation was mainly used as the test object and isolated rabbit's intestine and frog's rectus preparations were used only in confirmatory tests. Although only acetylcholine and serum was used in these estimations, the method was open to the criticism that response of the frog's heart muscle was not specific for acetylcholine, and that the esterase of the cardiac muscle is playing an uncertain rôle.

It was therefore felt desirable to repeat the investigations using the isolated and eserinized rectus abdominis of the frog, with a technique which would enable one to estimate the acetylcholine concentration of the substrate within a few seconds of mixing with the enzyme. Simultaneous estimations were made by means of the Straub heart technique.

EXPERIMENTAL.

Every effort was made to maintain constant conditions, the main variable factor being room temperature which affected the sensitivity of the preparation. Ringer solution at a pH of 7·8 was used as diluent with 0·1 c.c. serum and substrate made up to a final volume of 4·1 c.c. Acetylcholine hydrochloride (Roche) was used throughout.

During the autumn months with room temperatures of approximately 19°C. the strength of substrate was 1×10^6 and contraction was maintained for 1 minute. In the winter and spring good responses were obtained with concentrations of $1 : 10 \times 10^6$ although as the season advanced, 3 minute contractions were found necessary. Despite this change in sensitivity, no discrepancy could be discovered between estimations made simultaneously by different workers with separate preparations, or on consecutive days using the same sera.

Individual estimations were conducted by placing carefully without mixing, in the bottom of a beaker, 0·1 c.c. serum, together with 1 c.c. of substrate. At a suitable moment, by a vigorous rotation of the beaker, the enzyme and substrate were rapidly mixed, and allowed to react for varying periods of time at the end of which action was stopped by the rapid addition of 3 c.c. of eserine in Ringer solution again with vigorous mixing. All estimations were made in duplicate.

The final concentration of acetylcholine was estimated immediately by bracketing the muscular contraction obtained beween those obtained from two known concentrations in the usual manner (Fig. 1). By this means it was possible to determine the residual acetylcholine after the

[1] Sustained by a grant from the Scientific Research Endowment Fund of the Council for Scientific and Industrial Research.

enzyme had acted upon the substrate for 5 seconds and at any selected interval thereafter. In practice we made use of intervals of 5 seconds up to 30 seconds and then of 10 second intervals up to 1 minute. Owing to the fact that the rectus muscle loses its quantitative response after some forty contractions we abandoned attempts at using smaller time intervals, and in any case there was no advantage to be secured except perhaps in shortening the initial 5 second intervals. This had been reduced to 4 seconds but error in determining the actual time of mixing begins to assume relatively high proportions at this stage.

It was found that 1: 500,000 Prostigmine (Roche) could be used with advantage to replace eserine in stopping the reaction between enzyme and substrate, for it does not undergo decomposition like the latter (Hicks and Mackay, 1937).

DISCUSSION.

Three cases of myasthenia gravis, five cases of myotonia congenita and one case of possible periodic paralysis, together with twenty "normal" cases, were studied by means of the foregoing technique during some eight months, the pathological cases, except that of periodic paralysis, from which only three specimens of blood were obtained, on many different occasions.

In all instances the greatest rate of change in the substrate occurred as might be expected, during the first 20 seconds after mixing with the serum, but the greatest difference in rate of destruction caused by different types of sera was manifest in the first five seconds. If an examination is made of the composite diagram (Fig. 9) in which percentage of destruction of ester is plotted against time in seconds, it will be noted that at this early stage of reaction the sera fall into three distinct groups.

The greatest activity is shown by sera from the case (?) of periodic paralysis which causes a 32 p.c. hydrolysis in five seconds. Close to this follow the sera of the myasthenic cases with 20–29 p.c. destruction in five seconds. The normal sera cause hydrolysis of 8–14 p.c. in this same time interval, whilst the myotonia sera cause no measurable hydrolysis within five seconds of mixing. It is assumed that the accuracy of the method is about 5 p.c. (Chang and Gaddum, 1933), so that all that can be said is that any destruction of acetylcholine which does occur lies outside the limits of the method in these latter cases. Feldberg (1937) is of the opinion that an accuracy of no more than 10 p.c. can be expected, but our results have been too consistent to support this view.

The administration of quinine to these cases of myotonia had the apparent effect of measurably increasing the rate of destruction of acetylcholine in this early period of reaction. We have the impression that this difference is a real one, although owing to the limits of the method no accurate conclusion can be drawn. Addition to myotonic serum *in vitro*, of similar amounts of quinine calculated on the basis of body weight treated as 85 p.c. water, gave rise to no measurable alteration in the rate of hydrolysis.

It is obvious on inspection of the figure that after 15 seconds the differentiation of the sera into groups becomes less clear-cut and the upper and lower limits of normal rates of hydrolysis begin to merge with those of the less extreme pathological sera.

Stedman and Russell (1936) reported that sera from some cases of myasthenia gravis showed a reduction in choline-esterase activity as compared with that of "normals"; whilst McGeorge (1937) using a method based on that of Stedman and White (1933) reports extreme variability in the esterase activity of "normal" sera, within the range of which fall the rate of hydrolysis produced by sera from myasthenic cases. The application of those methods which utilize chemical means for determining the degree of hydrolysis of acetylcholine are necessarily limited to such a stage in the reaction that adequate quantities of acid or of displaced carbon dioxide are present for quantitative estimation.

TABLE 1.

Protocols of the Experiments Illustrated in (Fig. 9) only.

				5	10	15	20	25	30	40	50	60 Seconds	
5. 5.37	P	periodic	32	44		56		60	64	68	72% destruction	
10. 5.37		paralysis		32		44		48	50	56		„
4. 9.37	S	myasthenia gravis	20	28	28–32	44	44–48	48–50	52–56			„
4. 9.37	W	„	29	31–34	38	38–44	45	50–53	60			„
17. 9.37	T	„	20–23	23–28	28	33	38	38–42	45–48	45–48	48	„
19. 5.37	J	myotonia congenita	0	4		12	16	20	24		28	„
26. 5.37	J	„	0	11·2	20	24·8		27·2	31·2	36·8		„
13. 7.37	T	„	0	17·2								„
15. 7.37	T	„	0	4·8	20	24·8		28–32	32–36	40	44	„
28. 9.37	T	„	3	10	13	20						„
13. 7.37	B	„	0	12	20	24–28	24–28	28–32	32–36	36–40	41·2	„
3. 8.37	B	„	0–4	12–16	16–20	20–24	28					„
28. 9.37	B	„	3–5	5–10	13–17	17–20						„
2.10.37	H	„	0–5	5–9	9–13	17–20	20	20	23–27	27–30		„
2.10.37	H (2)	„	5–9	9–13	13–17	17–20		23–27	27–30			„
Normal sera.												
16. 4.37		normal	9	18	21	23	27	30				„
3. 5.37	N	normal	8	28		33·6		36·6	40	44	45·5	„
18. 5.37	N	T.B.	8	12	20	24		28				„
18. 5.37	N	T.B. (ii)	12	16		20		28				„
6. 6.37		normal	8	16·4	28	36		40				„
9. 6.37		gastric ulcer	8	13	17	20		23				„
23. 6.37		peptic ulcer	12	28·4	31·2	32·6		36				„
28. 6.37		normal		17	20	23			25	32	34	„
7. 7.37		fracture	10	17	20	25		40	44			„
18. 8.37		normal	16	24	28–32	32–36		36–40	40			„
14. 9.37		fracture	9–14	17	17–20	20–23		29–34	38–41	41–45	50–52	„
24. 9.37		fracture	10	13	17–20	23–27	29	34	34–38	41	41–45	„
27. 9.37		fracture	13	17	20	29	38	38–41	41–45	45–47		„
1.10.37		fracture	10–13	13–17	17–20	20–23						„
21. 5.37		normal	12	16	20	20		28				„

The use of biological test objects enables one to deal with physiological concentrations of substrate, and after periods of hydrolytic activity which at least approach those which must necessarily be involved in any possible hydrolysis and resynthesis of acetylcholine by the tissues during the phenomenon of transmission of the nervous impulse.

An examination of the tracings, protocols and curves derived from these will

convey better than description the results obtained in typical instances. Some results obtained from interesting cases by means of the frog's heart technique are included together with the corresponding results obtained with the isolated rectus abdominis. It will be seen that the conclusions drawn in the previous paper (Hicks and Mackay, 1937) are substantially confirmed. The addition to the Straub cannula of 0·1 c.c. serum, together with 1 c.c. of a suitable dilution of acetylcholine (determined by the sensitivity of the preparation) showed in the cases of myotonia congenita a consistent delay in recovery from inhibition (Fig. 6), whilst the case of periodic paralysis showed the reverse effect (Fig. 7).

We have indicated the existence of some doubt concerning the validity of the diagnosis of periodic paralysis in this case because (1) there was no family history to corroborate it, and (2) apart from a history of typical symptoms on previous occasions, he was seen in a typical seizure only once, and before a consultant could be called in had largely recovered except for a manifest neurotic aftermath. Typical, however, was the rapid pulse (120), the nocturnal attack and the rapid recovery. The serum from which the results here reported were obtained was drawn on the *day following the attack when the above resting pulse rate was counted*, but otherwise no symptoms except of pardonable apprehension were manifest. The upper half inch of a column of two inches of serum showed a pale flocculent appearance and on dilution there was a manifest and persistent Tyndall phenomenon. We have come to associate the extent of this latter phenomenon with the degree of myasthenia in our cases and merely record the observation without comment. Serum obtained from the periodic paralysis case weeks after return to normal avocation showed neither the flocculent layer nor on dilution, the light scattering, and the esterase activity fell within normal limits.

SUMMARY.

1. The sera from the blood of three cases of Myasthenia Gravis, five cases of Myotonia Congenita, and one posible case of Familial Periodic Paralysis have been tested for cholinesterase activity by means of the isolated rectus abdominis muscle of the frog.

2. The sera from cases of Myasthenia Gravis, and Periodic Paralysis show consistently higher cholinesterase activity than do normal sera, and the sera from cases of Myotonia Congenita show consistently lower cholinesterase activity than the lower limits of normal sera.

3. A method of using the isolated rectus abdominis muscle to estimate the choline esterase activity of serum within five seconds of mixing with substrate is described. Prostigmine (Roche) in a concentration of 1 : 500,000 was found to be more stable than and as efficacious as eserine, for stopping the hydrolytic action of the cholinesterase at any desired stage.

4. The addition of Quinine Sulphate in concentrations calculated from the dose found efficacious in treating Myotonia Congenita had no effect upon the rate of cholinesterolysis.

ACKNOWLEDGMENTS.

Our thanks are due to Dr. J. Coverrton, of Gawler, and Dr. Graeme Robertson, of Melbourne, for providing the cases of Myotonia; to Dr. C. T. C.deCrespigny, for the probable case of Periodic Paralysis, as well as to those members of the honorary staff of the Adelaide General Hospital, who have so willingly co-operated in providing Myasthenic sera.

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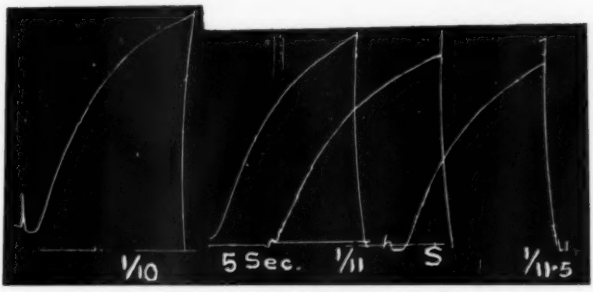
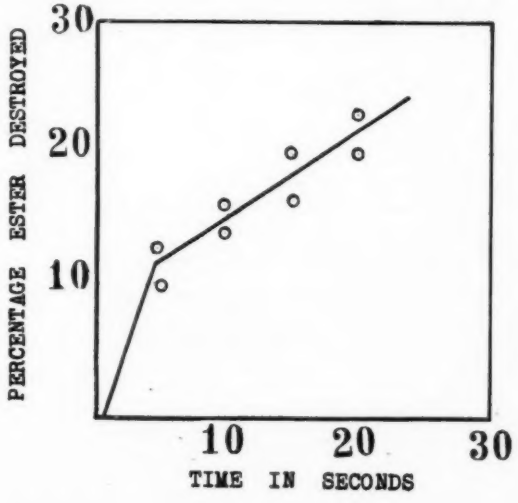


Fig. 1. Isolated eserinized rectus preparation to show amount of destruction of acetylcholine substrate in 5 seconds by normal serum. Standardizing $1/10^6$ acetylcholine; same $1/11 \times 10^6$; $1/11 \times 10^6$ + normal serum; $1/11{\cdot}5 \times 10^6$ acetylcholine.



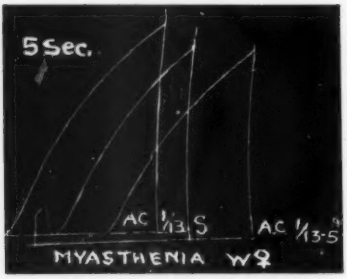
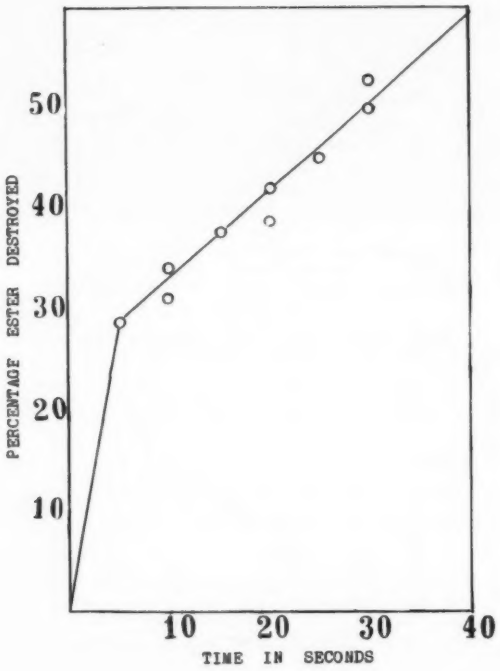


Fig. 2. Isolated eserinized rectus preparation. Myaesthenia case W. ♀. Standardizing acetylcholine 1/13 × 10^6; same substrate plus patient's serum for 5 seconds; Acetylcholine standard 1/13·5 × 10^6.



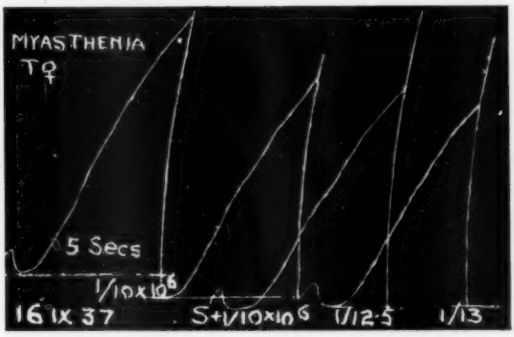
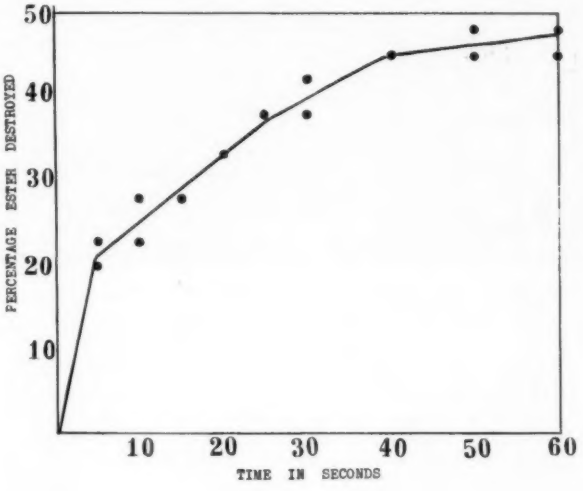


Fig. 3. Myasthenia case T ♀. Isolated eserinized rectus preparation. Acetylcholine $1/10 \times 10^6$; same substrate with patient's serum for 5 seconds; standardizing solutions $1/12{\cdot}5 \times 10^6$ and $1/13 \times 10^6$.



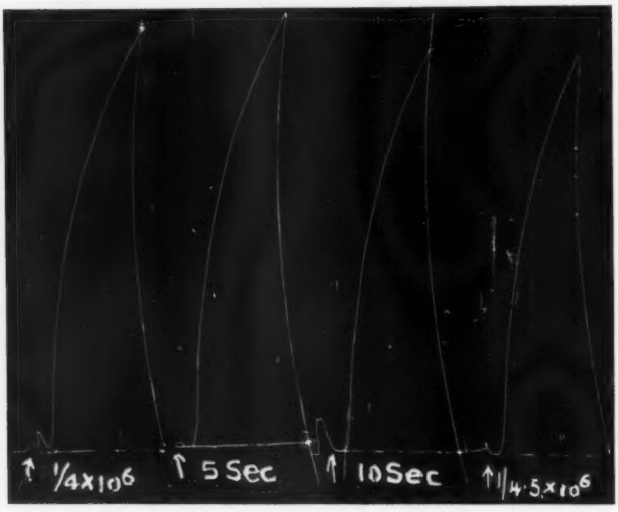
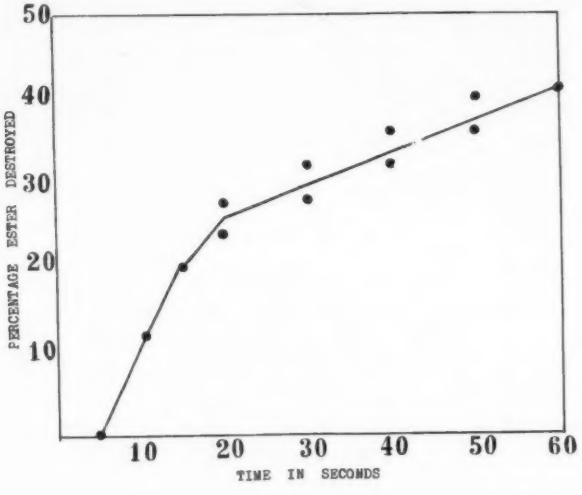


Fig. 4. Myotonia case B ♀. Standardizing acetylcholine $1/4 \times 10^6$, same strength substrate + patient's serum for 5 seconds; same + patient's serum for 10 seconds. Standardizing acetylcholine $1/4{\cdot}5 \times 10^6$.



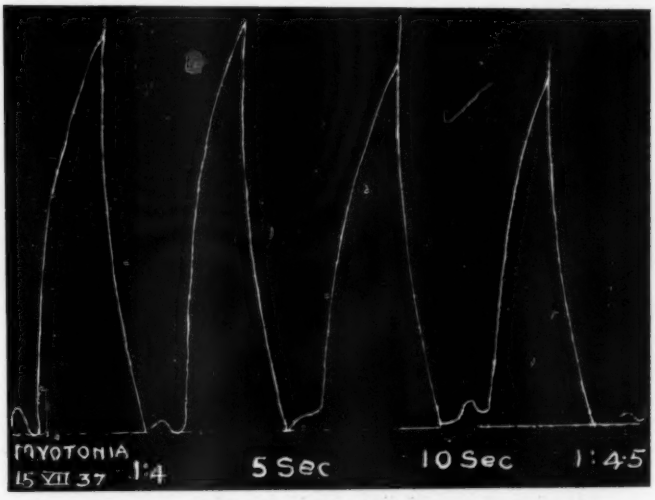


Fig. 5. Myotonia case T ♂. 15/vii/37. Isolated eserinized rectus preparation. $1/4 \times 10^6$ acetylcholine standard; the same 5 seconds after mixing with serum; same after 10 seconds; $1/4{\cdot}5 \times 10^6$ standardizing acetylcholine.

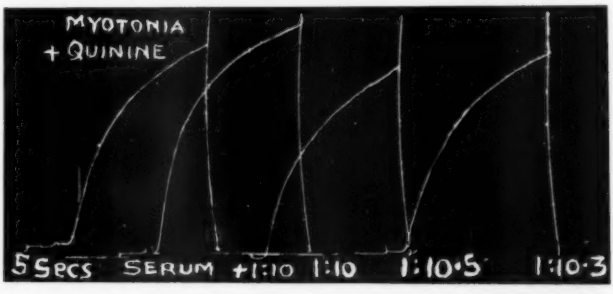
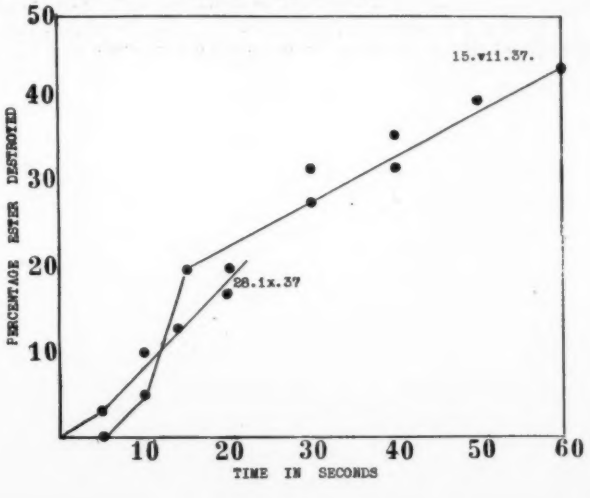


Fig. 5. Myotonia case T ♂. 28/vii/37 after 3 weeks treatment with Quinine Sulphate. Patient's serum + $1/10 \times 10^6$ acetylcholine; $1/1 \times 10^6$ acetylcholine standard; $1/10{\cdot}5 \times 10^6$; and $1/10{\cdot}3 \times 10^6$ acetylcholine standards respectively.



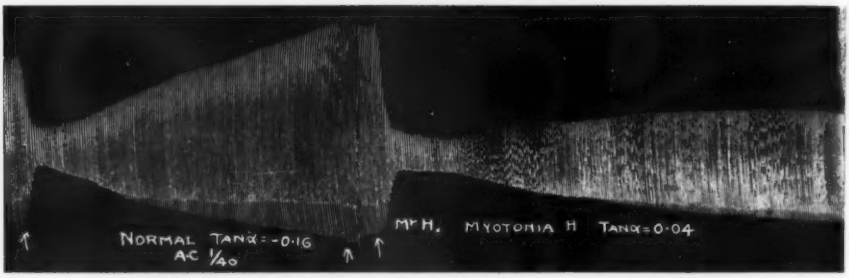
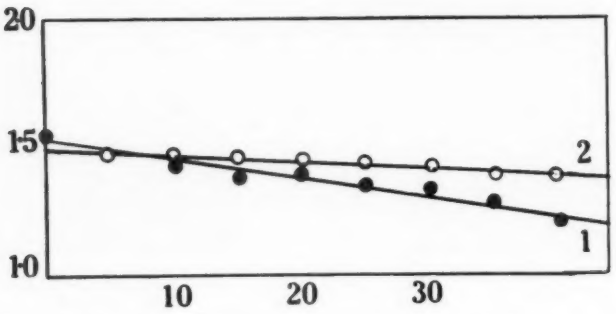


Fig. 6. Straub heart preparation. Normal serum and serum from Myotonia case H ♂, with same concentration of acetylcholine as substrate after.



Straub frog's heart preparation. Results of estimation of hydrolysis of acetylcholine substrate by sera from (1) Normal and (2) Myotonic (Thomsen) subject. Co-ordinates: Log $(S_\infty - S_n)$; Abscissae: tine 1. tan $\alpha = -0{\cdot}16$. 2. tan $\alpha = -0{\cdot}04$.

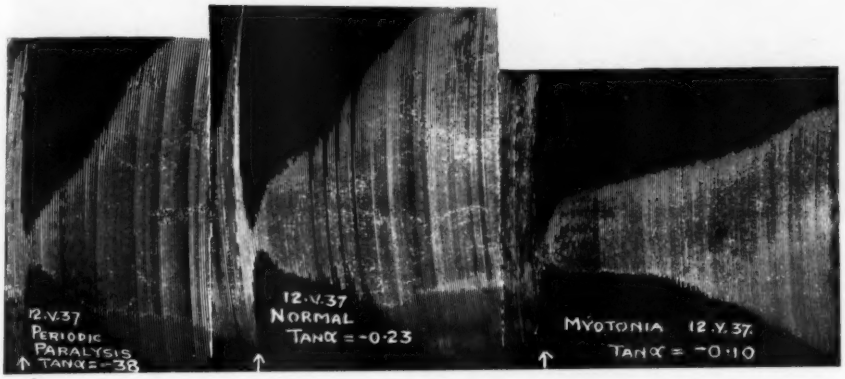
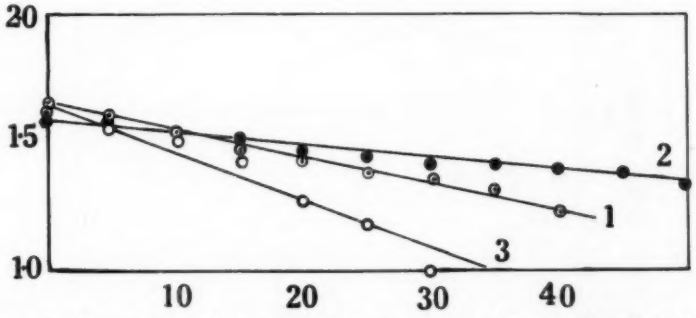


Fig. 7. Straub Heart preparation showing relative rates of destruction of same concentration of acetylcholine substrate by sera from cases of (?) periodic paralysis, myotonia congenita, and from a normal subject.



Straub frog's heart preparation. Results of estimation of rate of hydrolysis of acetylcholine substrate by (1) normal, (2) myasthenic, (3) (?) periodic paralysis subjects. Coordinates: Log ($S_\infty - S_n$). Abscissae: time 1. tan $\alpha = -0{\cdot}23$. 2. tan $\alpha = -0{\cdot}10$. 3. tan $\alpha = -0{\cdot}38$.

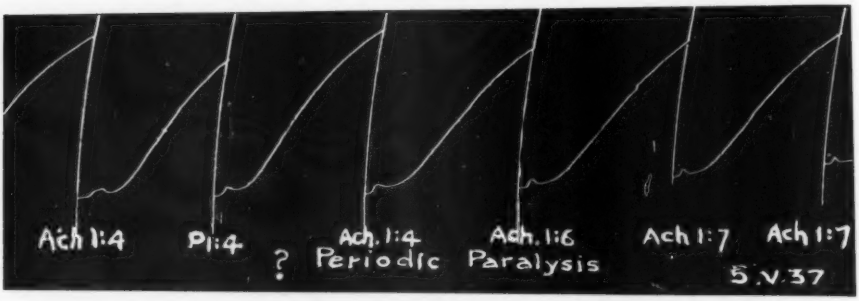
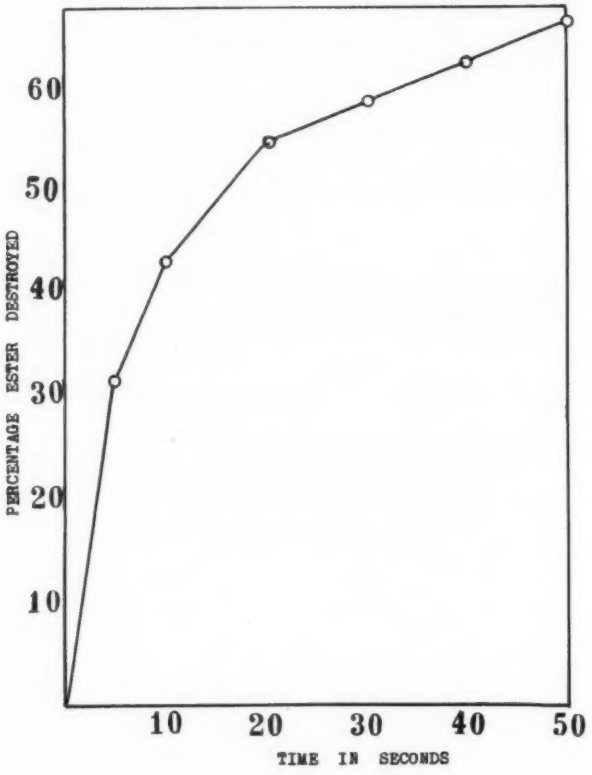


Fig. 8. Isolated eserinized rectus preparation. Acetylcholine standard $1/4 \times 10^6$; same substrate plus serum from (?) case of periodic paralysis for 5 seconds; $1/4 \times 10^6$ standard acetylcholine; standard $1/6 \times 10^6$; standard $1/7 \times 10^6$.



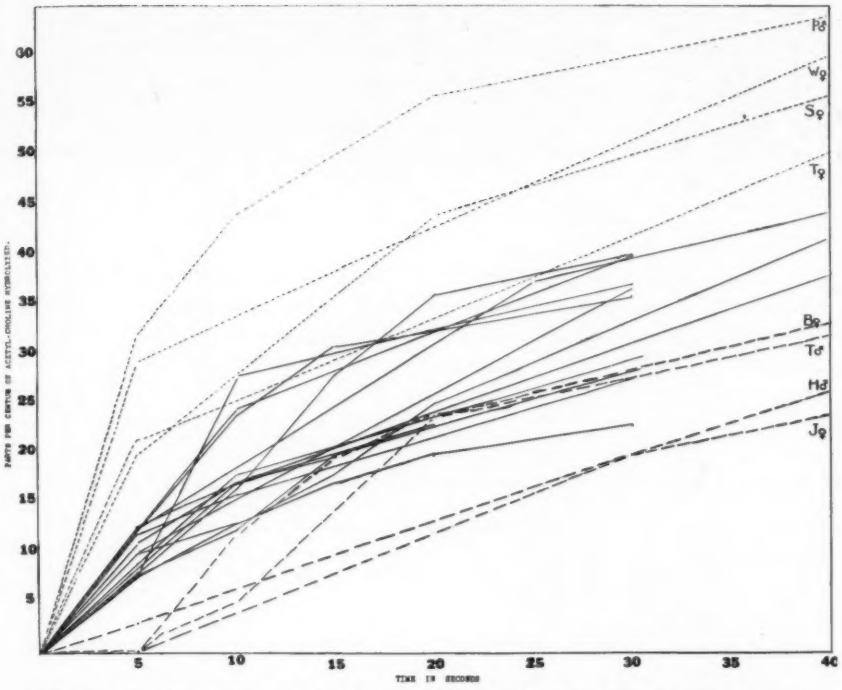


Fig. 9. To show relative rates of destruction of acetylcholine by sera from cases of Myasthenia Gravis (upper group) including one probable case of Familial Periodic Paralysis (P), from Normal subjects (middle group), and from cases of Myotonia Congenita, or Thomsen's Disease (lower group). To avoid crowding the diagram, repeated experiments on pathological sera have been omitted, as they almost invariably overlie each other.

MYXOMA AND THE SHOPE FIBROMA

4. THE HISTOLOGY OF SHOPE FIBROMA.[1]

by

E. WESTON HURST

(From the Institute of Medical and Veterinary Science, Adelaide, South Australia).

(Submitted for publication 5th January, 1938.)

Both Shope (1932) and Andrewes (1936) have written briefly concerning the histology of the Shope fibroma. The following rather more detailed account resulted from an attempt to determine the precise nature and potentialities of the fibroma cell, with a view to future work on the relation between fibroma and myxoma.

EXPERIMENTAL WORK.

Six rabbits were inoculated intradermally in multiple areas, 3 with the OA and 3 with the IA strain of virus (Andrewes, 1936) for which I am indebted to Dr. C. H. Andrewes; excisions were practised at frequent intervals to furnish a graded series of specimens from the 1st to 108th day, with particular emphasis on the 1st to 14th day. Eighteen rabbits were inoculated intratesticularly, 9 with each strain of virus, and the testicle excised at the 4th to 35th day.

OA virus.

Intradermal inoculation.

The first appearance, size, sharpness of demarcation, redness, moisture and duration of the tumour vary with the virulence of the virus and the breed of rabbit; other things being equal, Angora rabbits give a better reaction than do other breeds. The following appearances are those produced by a virus highly virulent after serial intratesticular passage.

After 2 to 3 days a slight pink flush at the site of inoculation rapidly develops into a definite swelling, which by the 6th day is bright red; the colour fades temporarily on anaesthesia or other disturbance of the animal. The most intense reactions later take the form of large diffuse plaques, and smaller secondary nodules may develop along the lymphatics or deep to the superficial muscle. On section the skin is highly translucent and oedematous, especially in its deeper layers, and a mucinous fluid less abundant than that in myxoma exudes. By the 9th to 13th day vesiculation may be marked in the overlying skin, or the centre of the tumour may be necrotic and purplish in tint; with multiple tumours the surrounding veins may now be greatly dilated. Thereafter the centre of the tumour may slough away, with resultant sepsis hidden by a dry scab, or the centre may liquify completely and on puncture yield a sticky fluid containing no polymorphonuclears

[1] Papers 1 to 3 of this series were published in the British Journal of Experimental Pathology, Vol. 18. The inoculations mentioned in the present paper were mostly performed at the Lister Institute, London.

but only isolated degenerate tumour cells. Where little or no necrosis occurs, the tumour becomes brownish or yellowish in colour. Regression may begin in about 12 and terminate in about 25 days, or with multiple tumours which have led to debilitation of the host may be delayed and take as long as 171 days; in the latter event the virus can be detected for at least 108 days.

Microscopical appearances. On the 1st day after inoculation the histological changes cannot be distinguished from those evoked by normal testicle. On the 2nd day they are similar but slightly more intense.

By the 3rd or 4th day vascular congestion is marked, cellular increase more evident, and oedema and fibrinous exudate already considerable. In the affected areas many polymorphonuclear leucocytes are present, together with numerous histiocytes. The connective tissue cells are more

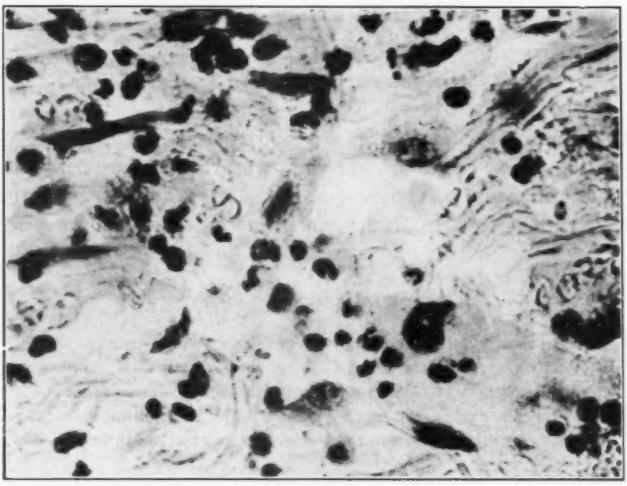


Fig. 1. OA virus. 3 days after intradermal inoculation. Inflammatory reaction and some enlargement of connective-tissue cells.*

numerous than normal, and somewhat enlarged in both body and nucleus (Fig. 1). Some leucocytes are karyorrhectic, and occasional fibroblasts necrotic. The adventitial and endothelial cells of the capillaries and venules are overactive. The epithelium may show numerous mitoses, and a few nuclei of the Malpighian layer punctate hyperchromasia.

By the 6th day the local fibroblasts are proliferating very actively and show numerous mitoses; they lie in columns two or three deep between the existing collagen bundles of the dermis. They are now much larger than those seen commonly in a healing wound. While most are elongated or stellate with markedly fibrillar protoplasm, a few similar cells already possess enormous, more or less rounded, and often vacuolated cell bodies. Their nuclei are less vesicular, and less hyperchromatic at the periphery than is the case in myxoma (Fig. 10); the cells are much more numerous and individual cells much larger. Nevertheless there is a distinct resemblance between the pathological picture of the two maladies. The nucleolar-nuclear ratio of the fibroma cells is low. Many such cells show oxyphilic masses in their cytoplasm; these often appear to be the degenerating remnants of phagocyted or invading leucocytes. Fibrinous exudate is very intense, and

* All photographs were taken at the same magnification and are from preparations stained with haematoxylin and eosin.

with the other inflammatory changes extends between the bundles of superficial muscle into the subcutaneous tissues. Swelling and proliferation of the endothelium of the capillaries and venules, and proliferation of the adventitia of the smaller vessels are now more pronounced, though much less marked than in myxoma. The adventitial proliferation frequently gives rise to foci of fibroma cells of unusual density and smaller than those elsewhere. Vast numbers of karyorrhectic polymorphonuclears are present in many cases.

By the 8th to 9th day the central parts of the lesion show massed karyorrhectic polymorphs and comparatively few tumour cells, while more peripherally fibroma cells are abundant with a mainly mononuclear exudate (Fig. 2). Pre-existing collagen bundles are now widely separated. In the overlying epithelium a few small vesicles are present in places, while in other areas some proliferation has taken place; at this stage the hair follicles are less obviously affected than later.

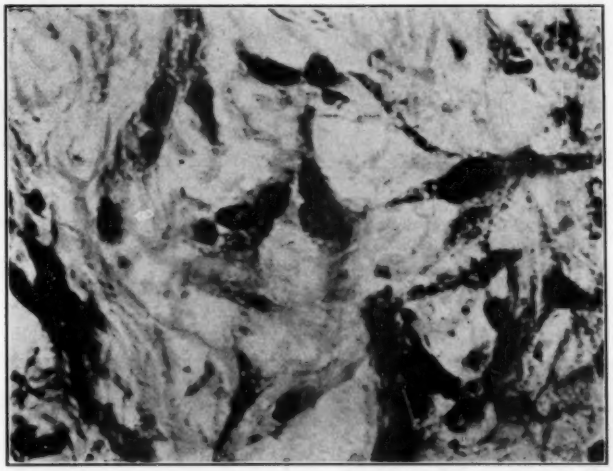


Fig. 2. OA virus. 9 days after intradermal inoculation. Enlarged and stellate fibroma cells widely separated by fibrinous exudate.

The formation of vesicles is preceded by punctate hyperchromasia or pyknosis of the nucleus of a cell or cells; the cell bodies then become ballooned, with their protoplasm represented only by isolated granular oxyphilic clumps. The resemblance to myxoma is again obvious.

By the 13th to 17th day, regression having usually set in, the edge of the tumour has become clearly defined from its surroundings. Peripherally, intense mononuclear and polymorphonuclear infiltration accompanies moderate swelling and multiplication of local fibroblasts, and the collagen bundles lie close together. Through a brief intermediate zone these appearances give place abruptly to a central area where large and stellate fibroma cells are widely spaced with little intervening collagen. The lack of collagen is due in great part to the separation of existing bundles by the exudate and new-formed structures, though possibly there is also some removal. Centrally in this area fibroma cells are less abundant than at the growing edge, and a few may appear necrotic. In places they are lining up, and are linked by their cytoplasm apparently to form new vascular channels (Fig. 3). The fibroma may extend through and separate bundles of the superficial muscle, a few fibres of which may be degenerate. In foci, polymorphonuclears may still be very numerous; some lie in vacuoles in the cytoplasm of fibroma cells. In the overlying epithelium vesiculation is well marked, and in the hair follicles many epithelial cells now show

Fig. 3. OA virus. 13 days after intradermal inoculation. The cells lie more closely together. In the centre they are lining up, apparently to form a capillary blood vessel. Note the large cell above and to the left.

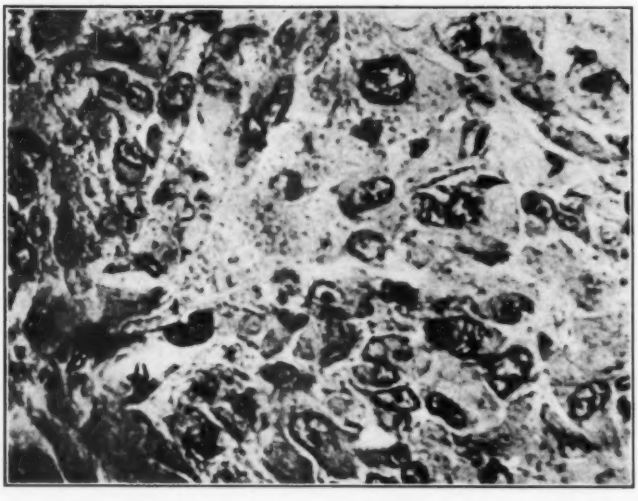


Fig. 4. OA virus. 23 days after intradermal inoculation. The cells are becoming rounded and lie closely together.

cytoplasmic changes very like those in myxoma, and consisting in the development of clumps of acidophilic granular material in an otherwise empty cell; nuclear changes are, however, very much less severe than in myxoma.

At the 23rd to 30th day the remains of inclusions are still visible in cells of the hair follicles. Some follicles are partly disorganized, and with the underlying fibromatous tissue may be wholly necrotic. Of the superficial epithelium only the Malpighian layer may survive, or even this may be necrotic. In one case without necrosis there was considerable warty thickening of the epithelium, with much elongation of its processes into the deeper tissues; in this case inclusions

Fig. 5. OA virus. 44 days after intradermal inoculation. The cells are rounded and inactive; no collagen fibrils were present between them.

were especially abundant. A far greater proportion of fibroma cells have swollen, irregular, polygonal or rounded bodies with one or more nuclei (Fig. 4), and only deep down are actively growing stellate cells present. Here advance of the fibroma often appears to be limited mainly by progressive condensation of the connective tissue, rather than by a marked inflammatory reaction as in the dermis. From now onwards the fibroma cells come to contain one or more protoplasmic inclusions, apparently products of degeneration, of either granular or completely hyaline structure and neutrophilic to strongly oxyphilic in reaction. The largest inclusions attain a diameter of 20μ. The cells now lie much closer together, due to the absence of oedema and exudate, and on an average are probably smaller than previously. In most parts of many tumours there is little or no new-formed collagen, though a few cells are laying down delicate strands; any apparent increase is due almost wholly to closer approximation of collagen bundles originally present. Vascular changes have subsided.

At the 39th to 44th days appearances are essentially the same (Fig. 5). Regeneration is occurring in the superficial epithelium. Deep down, the now rounded fibroma cells are very small and crowded together. In some places where necrosis occurs the tumour cells may develop into multinucleated giant cells.

At the 53rd to 68th days inclusions in the epithelium appear as oxyphilic masses near the nuclei of normal cells. It is not certain whether this appearance indicates the incorporation in normal cells of the dead remains of affected cells, or whether it points to the recovery of affected cells. All the cells of the fibroma are rounded and close together, and inclusions in their protoplasm larger and more numerous. Newly formed collagen is absent from most regions.

In tumours persisting for 108 days all cells may be rounded, and odd ones can be seen to be necrotic and surrounded by leucocytes (Fig. 6). Polymorphonuclear infiltration may still be marked, even in the absence of ulceration. Protoplasmic inclusions are still more abundant.

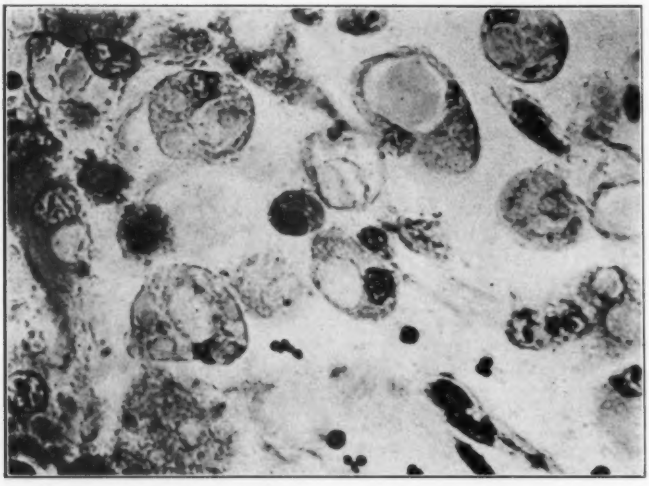


Fig. 6. OA virus. 108 days after intradermal inoculation. The rounded cells contain larger and smaller hyaline inclusions, probably degenerative in nature. Note the polymorphonuclears still present. There was no new-formed collagen in this preparation.

Intratesticular inoculation.

Allowing for differences in situation, lesions after intratesticular inoculation are essentially similar to the foregoing. They soon spread to the epididymis and fatty tissue at the head of the organ. Inflammatory changes are marked, as is swelling of the vascular endothelium and cells of the serosa.

By the 5th day the multiplying fibroblasts are already slightly larger than those in infections with the IA virus, and from the 7th day onwards the pathological picture is dominated by the proliferation of large fibroma cells derived from the vascular adventitia, interstitial connective-tissue cells, cells of the capsule, and so on. Sometimes fibroma cells may be found between muscle cells in the media of medium-sized arteries. The frequently whorled arrangement of these cells in the testis is due no doubt to the disposition of the local connective tissue.

It is quite clear that tubular degeneration is not due mainly to pressure from oedema and the proliferating fibroma cells. At an early stage, though not later, it is as severe with the IA strain of virus as with the OA. Spermatogenesis is arrested as early as the 6th or 7th day in many tubules, while in others bizarre multinucleated masses may be formed by abnormal proliferation

on the part of the germinal epithelium. Finally the seminiferous epithelial cells may show ballooning of their cytoplasm and acidophilic clumps very like those in the epidermis. These changes are not necessarily rigidly co-extensive with the earliest proliferation of fibroma cells. Necrosis first of individual cells and later of whole tubules follows, and now pressure from the proliferating fibroma cells no doubt becomes a factor in their destruction. Ultimately, normal structures are wholly replaced.

At the 4th to 21st day many fibroma cells are rounded or polygonal in form and often contain multiple nuclei; the proportion of such cells increases with longer duration of the lesions. There is possibly a greater tendency to the deposition of new collagen in the testis than in the skin. No staining occurs with muci-carmine. At these later periods (35 days) occasional

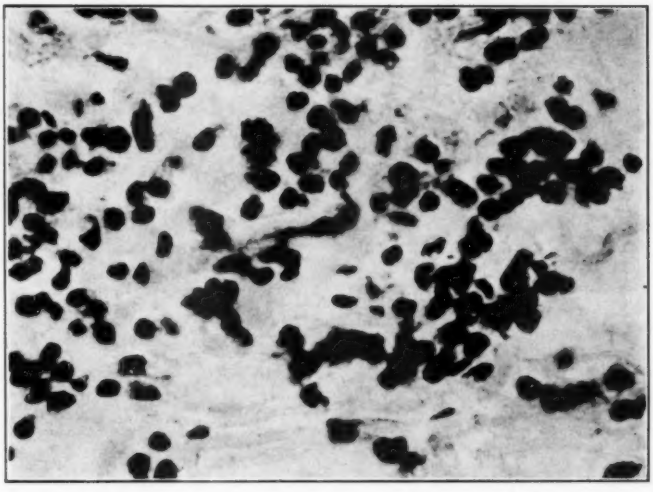


Fig. 7. IA virus. 9 days after intradermal inoculation. A dense and largely mononuclear exudate marks the lesion.

fibroma cells are still undergoing acute necrosis, this in the absence of sepsis which is often a contributing factor in the similar development in skin tumours.

In view of the demonstration of virus in the general viscera of rabbits at an early stage of infection (Hurst, 1937c), a careful search for lesions was undertaken in the earlier cases of this series. The local lymph nodes were somewhat swollen and showed in the sinuses an excess of large mononuclear cells. The spleen always showed changes typical of acute infection, but no characteristic lesions were found. In the liver and adrenals small mononuclear foci consisting of lymphocytes and enlarged endothelial cells were frequently present; without sufficient controls uninfected with fibroma virus, and in the absence of the characteristic proliferation of fibroblasts, the significance of these foci was difficult to assess. In one animal inoculated intravenously, however, such lesions were pronounced; in addition, in the lungs several areas of proliferation of fibroma cells occurred in association with the peribronchial lymphoid nodules and around some of the bronchial arteries, while perivascular and peribronchial cuffing with polymorphonuclear cells and lymphocytes was seen in other regions.

IA virus.

Intradermal inoculation.

The macroscopic reaction to the inflammatory strain appears a day or two later than with the tumour-producing strain and is less intense. At the 9th day the large pink papules are less translucent on section and exude much less mucinous fluid than do tumours due to OA virus. Vesiculation in the overlying skin is also less marked. Regression begins in about 12 days and is complete in about 18 to 20 days.

Microscopical appearances. As stated, lesions appear somewhat later than with the OA virus but at the 6th day the skin is the site of well-marked inflammation with relatively fewer polymorphonuclear cells and more mononuclear cells than in the above. Both histiocytes and fibro-

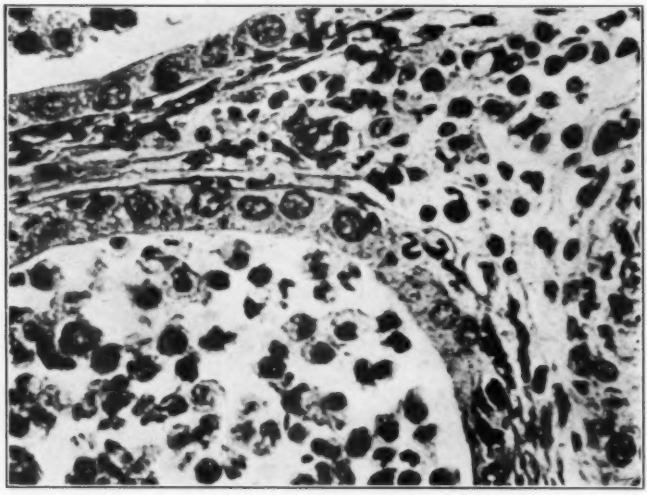


Fig. 8. IA virus. 9 days after intratesticular inoculation. The inflammatory exudate has spread between the ducts of the testis and the lumina are filled with degenerate cells instead of with spermatozoa.

blasts are definitely increased in numbers, but the latter are very much less numerous than with the OA virus and only rarely develop large forms comparable with those produced by this strain.

By the 9th day changes are more intense (Fig. 7), and necrosis and fibrinous exudate may be marked in the deeper layers of the dermis. The inflammation is spreading through the superficial muscle to the deeper tissues. Although occasionally oxyphilic clumping of the cytoplasm of some epithelial cells is seen, for the most part nuclear degeneration and vacuolation of the cytoplasm is the main abnormality in the dermis. A few cells show acute necrosis. At this stage the lesions differ from those due to the neuromyxoma virus (Hurst, 1937b) in the greater proliferation of fibroblasts, the much less extensive necrosis, and the relative sparsity of polymorphonuclears.

Later the inflammation recedes, and by the 16th day only slight residual changes are present.

Intratesticular inoculation.

In general the same differences as in the skin exist between the testicular lesions due to the OA and the IA virus.

A mainly polymorphonuclear exudate, less marked than with the OA virus, later gives place to mononuclear cells and eosinophils (Fig. 8). There is moderate enlargement and some multiplication of the connective-tissue cells. Spermatogenesis is arrested, and by the 14th to 21st day the tubules, though not necrotic, are mostly lined by a single incomplete layer of highly degenerate epithelium showing no mitoses. Much the same picture obtained on the 35th day, at the latest stage studied.

DISCUSSION.

Like myxoma (see Hurst, 1937 a) the Shope fibroma virus attacks both mesodermal and epithelial structures; the former are incited more to proliferation and the latter to degeneration. Despite obvious differences, there is an underlying similarity between the changes due to the OA virus and those of myxoma, though the more spectacular vascular and general lesions of the latter disease are wanting. After seventeen serial testicular passages at weekly intervals, without intermediate glycerination, neither the OA nor the IA virus had acquired the power of producing lesions more closely resembling those of myxoma.

Just as "myxoma" is now recognized to be a rather unfortunate name for the disease it denotes, so equally "fibroma" is not ideal. The tumours due to the OA virus cannot be regarded as true neoplasms, nor is deposition of collagen fibrils frequently a conspicuous activity of their constituent cells. Shope (1932) describes abundant collagen fibrils in both the natural and the experimental disease, but he does not state specifically that the fibroma cells are responsible for their formation. The present observations suggest that much of the collagen in the tumours may be pre-formed, and that many of the fibroma cells do not themselves deposit this substance. With the rapid enlargement of the tumour in its early stages, due in no small measure to abundant fibrinous exudate, existing collagen bundles are widely separated; as the exudative phenomena subside the size of the tumour diminishes markedly, and pre-existing fibrils, like the newly-formed fibroma cells, approach each other more closely. In the skin comparatively few of the fibroma cells lay down a few fine collagen fibrils; in the testis, apparently, the tendency to collagen formation is greater. The fibroma cell thus appears to be a connective-tissue cell (fibroblast, undifferentiated mesenchymal cell or sometimes perhaps histiocyte) stimulated to considerable proliferation and growth, only to lapse soon into a state of rounded inactivity which in many instances terminates sooner or later in degeneration and necrosis. In the skin, gross sepsis often destroys the tumour before the full cycle is run.

The proliferation of fibroma cells is beyond doubt determined by the virus, and if the foregoing observations are interpreted correctly the cessation of growth is, in a manner of speaking, predetermined. Virus can be demonstrated in persisting tumours for at least 108 days, yet the stimulus to growth has long since waned. At no phase is there a trend towards malignant neoplasia. The cytological characters of the cells are not those typical of malignancy. Attempts to

stimulate further growth of stationary tumours by incising the undersurface le only to more rapid regression. Transplantation of small pieces into other situation (muscle and liver), effective with the Shope papilloma in the hands of Rous an Beard (1934), here proved ineffective.

On the other hand the regression of the tumours does not seem to be brough about solely by the presence of antibodies in the blood; although such antibodie no doubt prevent the infection of fresh cells, a few mitoses in existing tumour cell are demonstrable well after antibodies have been formed. In experiments in

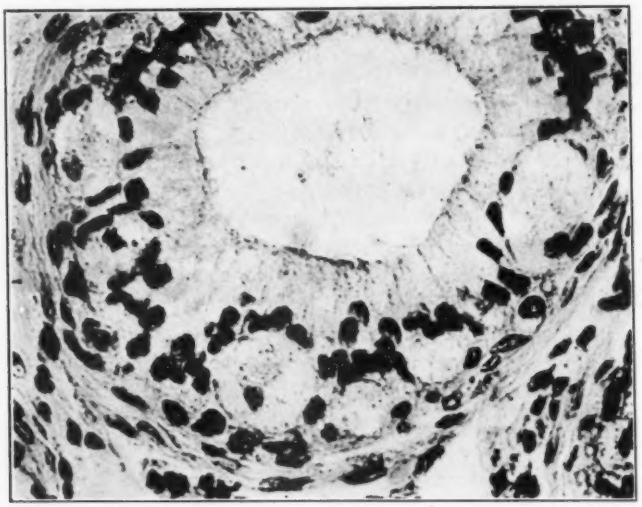


Fig. 9. OA virus. 10 days after intratesticular inoculation. Ballooned cells in epididymis with acidophilic granular material in place of normal cytoplasm.

which the rabbits were subjected to repeated general irradiation with X-rays before and after infection with OA virus (the actual tumour on the right flank was shielded with lead), the tumours were on an average larger than in controls but ceased to grow at almost the same time; regression, however, was prolonged greatly in many of the animals although abundant antibodies were present in the blood. The leucocyte count throughout these experiments was kept at about 1,000 to 2,000 white cells per c.mm.

While at first sight, therefore, the Shope fibroma presents certain resemblances to such virus-induced neoplasms as the Shope papilloma and filterable tumours of fowls, at the moment it seems improbable that it is in a true sense an

intermediate stage on the road to neoplasia, or anything more than a peculiar reaction to a virus infection.

In this connection recent observations by Fischer (1937) are of interest. If they are confirmed and the author's interpretation accepted, it cannot be expected that an intermediate process between non-malignancy and malignancy will ever be demonstrated in the form of a macroscopic lesion. Fischer believes that the malignant cell is a variety of the normal tissue cell, ubiquitous in the body, but probably too rare and too isolated to proliferate and form tumours. Various "realization"

Fig. 10. Myxoma (intradermal inoculation) on the 6th day for comparison with Figs. 2 and 3.

factors, including viruses, may induce multiplication of tissue cells and create an environment favourable to the proliferation of single malignant cells; when the number of these has reached a certain minimum, they may proceed to automatic independent growth.

SUMMARY.

As Andrewes recognized (1936) both OA and IA viruses evoke initially marked inflammatory phenomena at a site of inoculation; in lesions due to the latter, polymorphonuclears are relatively less numerous and mononuclears more numerous than with the OA virus. Later the IA virus causes also some proliferation of the local connective-tissue cells, which do not, however, approximate in number or in size to those found in infections with the OA virus. Both viruses

directly attack epithelial structures, whether in the skin or testis; in these structures destruction is more evident than proliferation. The relation to myxoma and the nature of the proliferation of connective-tissue elements in the fibromata are discussed briefly.

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PHYSIOLOGICAL ONTOGENY IN PLANTS AND ITS RELATION TO NUTRITION

4. THE EFFECT OF PHOSPHORUS SUPPLY ON THE TOTAL-, PROTEIN-, AND SOLUBLE-NITROGEN CONTENTS, AND WATER CONTENT OF THE LEAVES AND OTHER PLANT PARTS.

by

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(Submitted for publication 31st January, 1938.)

INTRODUCTION.

The first and second papers of this series (Ballard and Petrie, 1936, and Williams, 1936) were devoted to the consideration of the effects of nitrogen and phosphorus supplies on the growth of the plant and its parts; the effect of nitrogen supply upon the drifting composition of the leaves has been considered in the third paper (Petrie, 1937); and it is now proposed to take up the effect of phosphorus supply on the composition of the plant and its parts. Just as paper 3 is an extension of paper 1, so the present paper extends paper 2, and is based upon further data obtained from the two experiments on oat plants described therein. It should be emphasized that these studies have as their primary objective the elucidation of the integration of ontogenetic processes in growth, rather than an examination of phenomena associated with nitrogen and phosphorus deficiency.

The analytical methods adopted are identical with those described in paper 3.

DESCRIPTION OF DRIFTS.

In the following pages will be described some of the outstanding features of the drifts in the composition of the leaves in the two experiments. Data relating to other parts of the plant will also be considered, but mainly in so far as they

[1] This represents one of a series of investigations carried out at the Waite Institute and financed co-operatively by the Carnegie Corporation of New York, the Council for Scientific and Industrial Research, and the University of Adelaide.

throw light upon the course of the drifts in the leaves. The phosphorus treatments were as follows:

Experiment 1.

I 0·0093 gm. P per pot.
II 0·121 " " " "
III 0·486 " " " "

Experiment 2.

I 0·008 gm. P per pot.
II 0·09 " " " "
III 0·6 " " " "

Other experimental details are given in paper 2. For the sake of brevity the roman numerals I, II and III will be used to signify "plants of treatment I", etc.

Total Leaves.

Absolute drifts.

Experiment 1 (Table 1; fig. 1).

Protein Nitrogen.

Determinations were commenced at harvest 5, at which point the treatment relations are identical with those for dry weight. Thus the value for I is much lower than, and that for III slightly lower than, that for II. The parallelism between treatment relations of the absolute protein-nitrogen data and the dry-weight data continues for the next two harvests, but for the final three harvests the absolute values for protein nitrogen differ very little with treatment. A striking feature is that the maximum values for protein nitrogen occur at harvest 6 in II and III, and at harvest 8 in I. The dry-weight maxima for all treatments occur at harvest 8.

Soluble Nitrogen.

Except at harvest 5, where the picture is similar to that for protein nitrogen, treatment effects on soluble-nitrogen content are comparatively slight; nor is there any evidence for a delay in the maximum in I, the maximum in all plants occurring at harvest 7.

Water.

For harvests 2–8 the water content shows treatment relationships which are almost identical with those already described for dry weight. At harvest 9, however, the water content is the same in all plants, and at harvest 10 that for I is the highest. This feature is clearly an expression of the delayed senescence observed in I, and already discussed in paper 2. The maxima for II and III occur at harvest 7, thus being later than the corresponding maxima for absolute protein nitrogen

but earlier than the dry-weight maxima. The form of the curve for I closely resembles that of protein nitrogen; its maximum also occurs at harvest 8, and is delayed with reference to those of II and III.

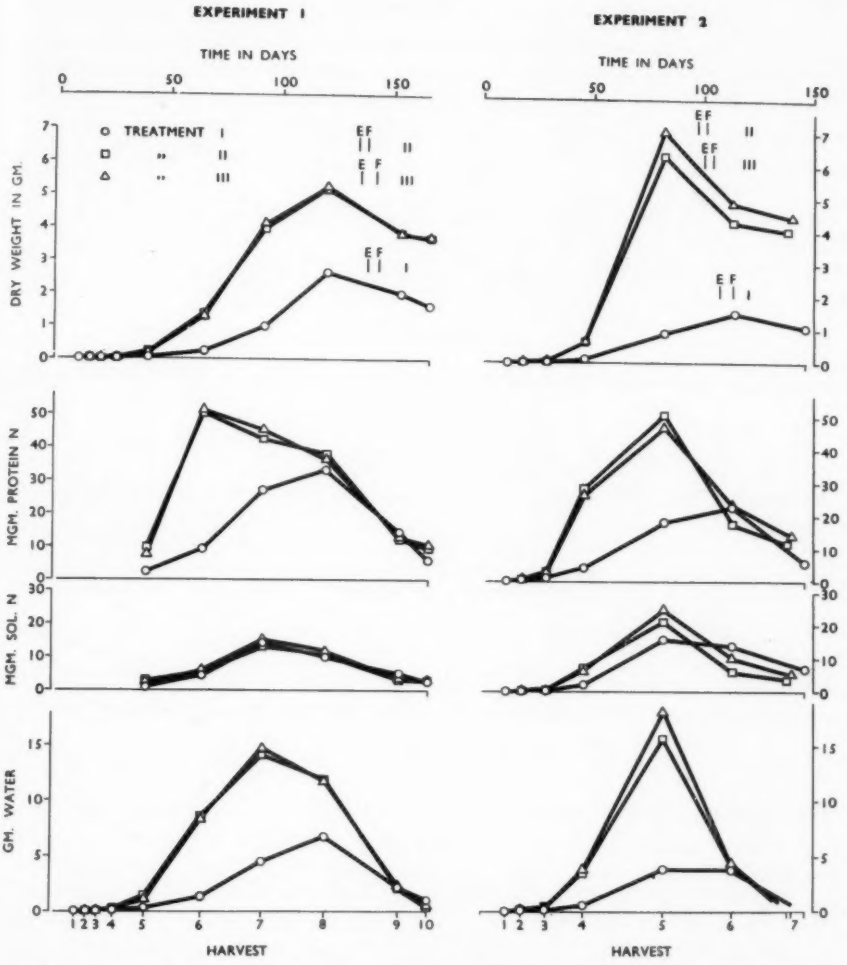

Fig. 1. Dry weight of and absolute amounts of constituents of total leaves per plant. From data of Tables 1 and 3. In this and subsequent figures, E indicates times of exsertion of inflorescences, and F times of flowering. At harvest 7, Experiment 2, the leaves were dead and their fresh weights were not taken.

TABLE 1.

Absolute Amounts of Protein Nitrogen, Soluble Nitrogen, and Water in the Leaves, Experiment 1.*

	Treatment I.			Treatment II.			Treatment III.		
Harvest	Prot. N.	Sol. N.	Water.	Prot. N.	Sol. N.	Water.	Prot. N.	Sol. N.	Water.
1			0·0195			0·0195			0·0195
2			0·0588			0·0671			0·0753
3			0·0782			0·114			0·109
4			0·119			0·267			0·227
5	2·37	0·98	0·343	9·82	2·72	1·47	7·47	2·11	1·08
6	9·20	4·34	1·37	50·4	5·5	8·59	51·1	6·0	8·34
7	26·7	14·1	4·53	42·3	13·0	14·1	45·2	15·1	14·7
8	33·1	10·0	6·74	38·1	10·6	11·9	36·3	11·8	11·8
9	14·5	5·1	2·18	12·2	3·3	2·09	12·8	3·4	2·22
10	5·96	2·50	1·00	9·36	2·81	0·397	10·35	2·79	0·492

* Amounts of water in grammes, and of nitrogen in milligrammes. per plant.

TABLE 2.

Percentage Amounts of Protein Nitrogen, Soluble Nitrogen, and Water in the Leaves, Experiment 1.*

	Treatment I.			Treatment II.			Treatment III.		
Harvest	Prot. N.	Sol. N.	Water.	Prot. N.	Sol. N.	Water.	Prot. N.	Sol. N.	Water.
1			726			726			726
2			656			652			674
3			596			729			693
4			531			648			588
5	4·04	1·67	585	4·79	1·33	717	4·67	1·32	670
6	3·93	1·85	584	3·60	0·39	615	4·02	0·47	658
7	2·70	1·43	459	1·07	0·33	356	1·10	0·36	358
8	1·26	0·38	258	0·74	0·20	231	0·69	0·23	224
9	0·73	0·26	111	0·32	0·09	55	0·34	0·09	58
10	0·37	0·16	64	0·25	0·08	11	0·28	0·07	14

* The values for water are the amounts associated with 100 gm. dry matter.
Water data: S.E. for means of 6 (harvests 2–8) = ± 10·1.
S.E. for treatment means of 12 (harvests 9–10) = ± 4·3.

Experiment 2 (Table 3; fig. 1).

Protein Nitrogen.

In general the picture is very similar to that obtained in Experiment 1, especially with regard to treatment effects. The maxima occur at harvest 5 for II and III and at harvest 6 for I, thus coinciding with the incidence of the dry-weight maxima. Comparison of the two sets of curves for II and III, however, reveals the probability that decrease in absolute protein nitrogen set in earlier than decrease in dry weight.

Soluble Nitrogen.

Treatment effects are much more pronounced than was the case in Experiment 1. Values for I are consistently low for harvests 2–5 and become the highest for harvests 6 and 7. There are also increases in the values for III as compared with II during maturity[2] and senescence. These increases are more marked than the corresponding ones in absolute protein nitrogen; in both cases they are associated with the fact that the dry weight of the leaves becomes significantly greater for III than for II during these growth stages. The maxima in soluble-nitrogen content all occur at harvest 5, but the form of the curve for I suggests a delay with respect to the other treatments.

TABLE 3.

Absolute Amounts of Protein Nitrogen, Soluble Nitrogen, and Water in the Leaves, Experiment 2.*

	Treatment I.			Treatment II.			Treatment III.		
Harvest	Prot. N.	Sol. N.	Water.	Prot. N.	Sol. N.	Water.	Prot. N.	Sol. N.	Water.
1	0·153	0·027	—	0·153	0·027	—	0·153	0·027	—
2	0·654	0·199	0·091	0·745	0·263	0·101	0·719	0·284	0·105
3	1·22	0·46	0·156	3·16	0·58	0·414	2·74	0·53	0·345
4	4·25	2·09	0·585	28·2	7·1	3·58	26·3	6·2	3·78
5	18·1	15·7	3·81	50·7	21·0	15·8	46·9	24·7	18·1
6	22·8	13·9	3·76†	17·6	5·9	4·16†	23·4	10·1	4·35†
7	5·88	6·9	—	11·8	3·8	—	14·1	5·3	—

* Amounts of water in grammes, and of nitrogen in milligrammes. per plant.

† The estimations of water content for harvest 6 are, through an accident, less reliable than those for previous harvests.

Water.

The picture is similar to that for Experiment 1, but the form of the curves suggests that loss of water from the leaves of I may precede loss of protein nitrogen.

Comparison of Experiments.

The second experiment differs from the first in that planting was seven weeks later; the range of phosphorus supply was also wider, and all plants received a slightly higher supply of nitrogen. The late planting shortened the growing period and, since the harvest intervals were comparatively large, the data of Experiment 2 do not so clearly illustrate treatment differences in the incidence of maxima or differences in the form of the drifts of individual plant constituents. The increased nitrogen level has tended to accentuate the differences between I and

[2] The custom adopted in previous papers of defining maturity as the stage of maximum dry weight is here continued.

II, and may also account in part for the observed increases in amounts of constituents present in III as compared with II. These increases are not demonstrable in Experiment 1.

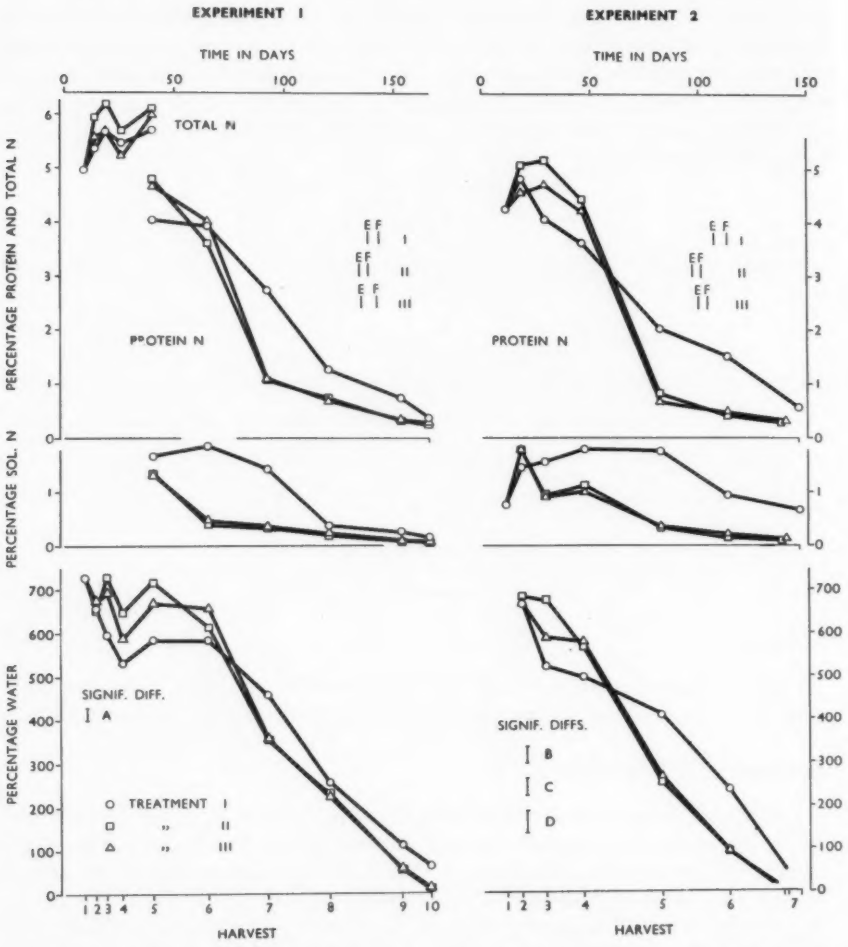

Fig. 2. Amounts of constituents of total leaves in gm. per 100 gm. dry matter. The significant differences for the water data are 2·828 × the standard error. A is for the standard error of means of 6 (harvests 2–8); B is for means of 6 (harvests 2–3; and harvest 4, treatment I); C is for means of 5 (harvests 4–5, treatments II and III); D is for a mean of 3 (harvest 5, treatment I). From data of Tables 2 and 4. At harvest 7, Experiment 2, the leaves were dead and their fresh weights were not taken.

RELATIVE DRIFTS.

Experiment 1 (Table 2; fig. 2).

Protein Nitrogen.

At harvest 5 the protein-nitrogen content is lowest for I but, on account of the rapid fall in the values for II and III, it becomes the highest at harvest 7 and at all subsequent harvests. The values for II and III are almost identical at all harvests.

Since no protein-nitrogen values are available for the first four harvests, the values for total nitrogen are plotted in fig. 2. These will be discussed in relation to the water-content data.

Soluble Nitrogen.

At all harvests the content of soluble nitrogen is highest in I and, as in the case of protein nitrogen, the differences between II and III are slight. With treatments II and III the soluble-nitrogen contents fall to a very low value even earlier than do the protein-nitrogen contents.

Water.

Analyses of variance were performed on the data for harvests 2–8 and 9–10 separately, interaction being significant in the first case and insignificant in the second. The standard errors are given in Table 2 and significant differences applicable to these and other sets of relative water-content data are indicated in the figures.

Apart from minor fluctuations the values remain fairly constant until harvest 6; after this there is a progressive fall with time with all treatments. At harvests 3–6 the water content in I is significantly lower than in II and III; thereafter the position is reversed and the differences are in most instances significant. The water contents of III are depressed below those of II for harvests 3–5 but there is a significant reversal at harvest 6. For the remainder of the growing period the values of II and III are almost identical.

In time drifts and treatment effects the protein-nitrogen and water contents resemble one another closely. For the early harvests the total-nitrogen data suggest that the relationship is present here also, especially if one assumes that the soluble-nitrogen content is increased in I at this time. The depressed values for both water and total-nitrogen contents at harvest 4 are of interest as an illustration of the effect of the environment on these quantities: the temperature was comparatively high and the humidity low on the day of harvest.

Experiment 2 (Table 4; fig. 2).

Protein Nitrogen.

Data for this experiment commence at an early stage of growth, and demonstrate a marked increase in protein-nitrogen content from I to II, especially at

harvests 3 and 4. In III, on the other hand, the values are definitely depressed below those for II at harvests 2 and 3. After harvest 4 the picture is very similar to that for Experiment 1.

Soluble Nitrogen.

There is an initial depression at harvest 2 in I, after which these plants have the highest content of soluble nitrogen throughout the experiment. For the later harvests the values are also higher than in the first experiment, doubtless because of the increased supply of nitrogen mentioned above. The differences in the values for II and III are again very small.

TABLE 4.

Percentage Amounts of Protein Nitrogen, Soluble Nitrogen, and Water in the Leaves, Experiment 2.*

	Treatment I.			Treatment II.			Treatment III.		
Harvest	Prot. N.	Sol. N.	Water.	Prot. N.	Sol. N.	Water.	Prot. N.	Sol. N.	Water.
1	4·25	0·76	—	4·25	0·76	—	4·25	0·76	—
2	4·81	1·46	668	5·07	1·79	685	4·55	1·80	667
3	4·07	1·56	524	5·16	0·94	676	4·70	0·91	590
4	3·63	1·79	499	4·44	1·12	568	4·21	1·00	580
5	2·03	1·76	410	0·81	0·33	255	0·67	0·35	268
6	1·52	0·93	238†	0·41	0·14	93†	0·48	0·21	93†
7	0·56	0·65	—	0·29	0·10	—	0·32	0·12	—

* The values for water are the amounts associated with 100 gm. dry matter.
† See footnote to Table 3.

Water data: S.E. for mean of 3 (harvest 5, treatment I) = ± 17·5.
S.E. for means of 5 (harvests 4 and 5, treatments II and III) = ± 13·5.
S.E. for means of 6 (harvests 2 and 3; and harvest 4, treatment I) = ± 12·3.

Water.

An analysis of variance was performed on the data for harvests 2–5; interaction was significant and the standard errors are given in Table 4. At harvest 6 the data are not very reliable, and at harvest 7 the leaves were dry.

At harvest 3 all treatment differences are significant, at harvest 4 the value for I is still below those for II and III, and at harvest 5 it is significantly above them. Once again there is a close relationship between the protein-nitrogen- and water-content data, especially with regard to the effect of treatment.

EARLY LEAVES OF PRIMARY SHOOT.

Experiment 1 (Tables 5 and 6; figs. 3 and 4).

ABSOLUTE DRIFTS.

Total Nitrogen.

The data for the first leaf do not extend beyond the point of maximum nitrogen content, although this point is probably closely approached in II and III, since the increments between harvests 3 and 4 are very small. At harvests 2 and 3

the value for I is the lowest, but at harvest 4 it approaches that for II; the values for II are the highest except at harvest 2. In the second leaf the nitrogen intake follows the increase in dry weight, the values for I being considerably lower than those for II and III. The data for the third leaf are more complete and show maximum nitrogen contents for II and III at harvest 5; thereafter there are considerable losses to other parts of the plant. The curve for I follows that for dry weight very closely and there is only a slight loss between harvests 6 and 7.

TABLE 5.

Absolute Amounts of Total Nitrogen and Water in the Early Leaves of the Primary Shoot, Experiment 1.*

	Treatment I.		Treatment II.		Treatment III.	
Harvest	Total N.	Water.	Total N.	Water.	Total N.	Water.
First Leaf						
1	0·133	0·0195	0·133	0·0195	0·133	0·0195
2	0·474	0·0588	0·598	0·0671	0·604	0·0753
3	0·684	0·0705	0·783	0·0882	0·713	0·0820
4	0·801	0·0695	0·811	0·0850	0·741	0·0750
Second Leaf						
3	0·061	0·0077	0·187	0·0258	0·179	0·0268
4	0·412	0·0482	1·27	0·149	1·05	0·125
Third Leaf						
4	0·009†	0·00097	0·265	0·0328	0·210	0·0262
5	1·08	0·137	2·41	0·279	1·95	0·214
6	1·78	0·163	1·70	0·296	1·75	0·260
7	1·49	0·144	0·17	0·025	0·14	0·018

* Amounts of nitrogen in milligrammes, of water in grammes, per plant.
† This value is calculated from the assumed relative value given in Table 6.

Water.

These curves more closely resemble those for dry weight than those for total nitrogen. The maxima for absolute nitrogen of the third leaves in II and III occur before those for water, but the senescent loss of water, as of nitrogen, is more complete than is the loss in dry weight.

RELATIVE DRIFTS.

Total Nitrogen.

There are quite marked effects of treatment upon the nitrogen contents of the first leaves even though these leaves were appreciably developed before the application of the nutrients. The value for I is low at harvests 2 and 3 but at harvest 4 it is the highest. The values for III are depressed below those for II at all three harvests. The second leaf values are very low for I, and once again those for III are depressed below those for II. There was insufficient material to obtain a value for the third leaf of I at harvest 4, but the value at harvest 5 indicates that the total-nitrogen content of I was probably low throughout the early stages of

growth. The values for II and III fall very rapidly after harvest 5 whereas those for I are maintained at a high level. The depression in III is less marked than is the case in the two earlier leaves.

TABLE 6.

Percentage Amounts of Total Nitrogen and Water in the Early Leaves of the Primary Shoot, Experiment 1.*

	Treatment I.		Treatment II.		Treatment III.	
Harvest	Total N.	Water.	Total N.	Water.	Total N.	Water.
First Leaf						
1	4·96	726	4·96	726	4·96	726
2	5·36	656	5·95	652	5·57	674
3	5·79	596	6·28	707	5·70	656
4	5·91	513	5·65	592	4·97	502
Second Leaf						
3	4·73	592	5·87	809	5·59	833
4	4·78	560	5·79	678	5·40	641
Third Leaf						
4	4·78†	561	5·45	673	5·22	651
5	5·38	679	6·20	712	6·08	667
6	6·15	562	3·57	625	4·24	634
7	5·64	545	0·64	93	0·68	85

* Amounts of water in grammes associated with 100 gm. dry matter.
† This value is assumed to be equal to that of second leaf at the same time, since material was insufficient for analysis.

Water data: First Leaf, S.E. for means of 6 (harvests 2–4) = ± 13·0.
Second Leaf, S.E. for means of 6 = ± 20·0.
Third Leaf, S.E. for means of 6 = ± 20·3,
S.E. for mean of 3 (harvest 4, treatment I) = ± 28·7.

Water.

Analyses of variance were performed on the data for the three leaves; interaction was significant in each case, and the standard errors for individual means are given in Table 6.

For the first leaf the water content of I is lower than those for II and III at harvest 3, and than that for II at harvest 4. There is also a depression in III with respect to II at harvests 3 and 4. In the second leaf the water content is significantly lowered in I, and this also holds for the third leaf at harvests 4 and 6. There are no significant differences between II and III for either the second or third leaves, and after harvest 6 the water content of the third leaves of II and III falls rapidly.

OTHER PLANT PARTS.

In fig. 6 are presented total-nitrogen data for the primary shoot in Experiment 1, and in Table 7 and fig. 5 similar data for the stems, roots and inflorescences of Experiment 2. These need not be described in detail here but will be referred to in the general discussion which is to follow.

Data of Other Workers.

A characteristic feature of the relative protein-nitrogen data of the present experiments is the reversal in the relations of I and II during the life-cycle. A similar reversal in the nitrogen content of the plant is shown in an experiment with varying potassium supply reported by Gregory (1937). In both cases the

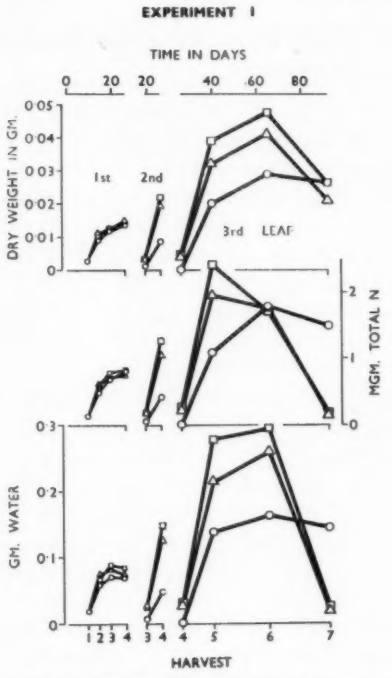

Fig. 3. Dry weights of the 1st, 2nd and 3rd leaves of the primary shoot and their absolute contents of total nitrogen and water. From data of Table 5.

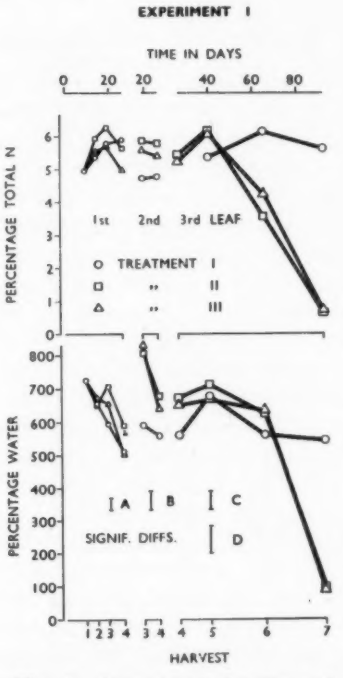

Fig. 4. Amounts of nitrogen and water in the 1st, 2nd and 3rd leaves of the primary shoot in gm. per 100 gm. dry matter. Significant differences A, B and C are for means of 6; A, first leaf; B, second leaf; C, third leaf; D, third leaf for harvest 4, treatment I (a mean of 3). From data of Table 6.

amount of growth made is evidently the determining factor. Strebeyko (1934) gives total-nitrogen data for the shoots of oats at five stages of growth and protein-nitrogen data for the first three of these; his plants were grown at five levels of phosphorus supply. At harvests 2–5 nitrogen content tended to decrease with

increasing phosphorus supply, but at the first harvest it increased up to the fourth level of supply and was depressed with the fifth. His protein-nitrogen values are excessively low but they demonstrate even more marked treatment effects at the first harvest. Both high and low levels of phosphorus tended to depress the protein content at this time; thus the data support the findings of this paper.

TABLE 7.

Absolute and Relative Amounts of Total Nitrogen in the Stems, Roots and Inflorescences, Experiment 2.

	Absolute Nitrogen in Milligrammes. Treatment			Nitrogen per cent. Dry Weight. Treatment		
Harvest	I.	II.	III.	I.	II.	III.
Stems						
1	0·432	0·432	0·432	3·79	3·79	3·79
2	0·305	0·436	0·446	4·06	5·19	5·13
3	0·400	1·22	1·15	3·54	5·07	5·08
4	1·38	10·6	11·0	3·45	4·19	4·03
5	10·8	32·5	32·7	2·56	0·66	0·66
6	25·4	38·6	41·0	0·90	0·30	0·31
7	13·9	14·9	15·6	0·48	0·14	0·15
Roots						
1	0·151	0·151	0·151	3·21	3·21	3·21
2	0·462	0·396	0·360	4·16	3·96	3·79
3	1·40	1·45	0·959	4·19	3·98	4·15
4	3·82	8·14	6·86	2·87	3·06	3·13
5	23·8	24·5	25·8	3·30	0·76	0·64
6	22·0	15·4	15·5	1·82	0·53	0·54
7	12·2	14·1	14·1	1·23	0·59	0·61
Inflorescences						
6	13·0	48·3	40·3	1·48	1·18	1·21
7	57·5	81·7	75·7	2·13	1·05	1·02

Richards and Templeman (1936)[3] demonstrate with phosphorus deficiency a marked reduction in protein-nitrogen content of successive leaves of barley. Their data suggest that the high content of soluble nitrogen found in the leaves of I in these experiments was probably due to an accumulation of amino- and particularly of amide-nitrogen.

Gilbert, McLean and Adams (1927), Jones (1936), Kraybill (1930), Kraybill and Smith (1924), McGillivray (1927), and Turtschin (1936) have all shown increases in relative contents of total soluble nitrogen or individual soluble nitrogen

[3] It is unfortunate that the data of these workers was not presented upon an absolute as well as upon a relative basis. Although each successive leaf was sampled first at the time of complete emergence, it is possible that it did not attain maximum dry weight until later; thus a fall with time in the percentage content of a given constituent may not mean a loss of the constituent from the leaf. In the experiments reported in the present paper net protein hydrolysis was delayed in the leaves of plants with low phosphorus supply, and it would have been of interest to know whether this also holds when the nitrogen supply is adequate at all stages of growth.

compounds with phosphorus deficiency, and some of them have also demonstrated an accompanying decrease in insoluble- or protein-nitrogen content. Dunne (1932) attributes an increase in the buffer capacity of the sap of plants with deficient phosphorus supply to increases in content of amides, amino-acids and sugars; he quotes Kraybill in support of this attribution.

DISCUSSION.

1. *The Initial Depression with High Phosphorus Supply.*

As in the case of nitrogen, discussed in papers 1 and 3, the largest application of phosphorus produced a temporary reduction in the growth of the plant and its parts. In the case of Experiment 2 this depression was shown in paper 2 to be entirely due to a brief but significant reduction in the unit leaf rate during early growth. The depression in dry weight was reflected in the absolute amounts of protein nitrogen, soluble nitrogen and water present in the leaves; there were also reductions in the percentage contents of protein nitrogen and water but not in that of soluble nitrogen. In Experiment 2 a significant depression in percentage water content did not occur until harvest 3 (fig. 2), whereas the protein-nitrogen content was considerably reduced at both harvests 2 and 3. Similarly in Experiment 1 the percentage nitrogen content was appreciably lowered before there was a significant depression in the water content. These facts suggest that the reduction in water content resulted from the treatment effect on nitrogen content rather than *vice versa*. The reduction in protein-nitrogen content and unit leaf rate can be attributed, therefore, only to an effect of the high external concentration of phosphate on the intake of nitrogen by the roots. If a high phosphorus content of the leaves were detrimental to protein synthesis one would expect an increase in the soluble-nitrogen content of the leaves with this treatment.

The data of Strebeyko (1934) do not show an initial depression with high phosphorus supply in the dry weight of the shoots, though, as already stated, there was a depression in their protein-nitrogen content. It has been shown in the present experiments that the depression in the dry weight of the roots of III was more pronounced than that of the leaves or stems; thus, on a whole plant basis, a growth depression may have occurred in Strebeyko's experiments. Neller (1935) also has demonstrated growth depression and injury as the result of applying acid phosphate manures to peat soils.

2. *Factors Governing the Amounts of Total- and Protein-Nitrogen in the Leaves.*

In the third paper of this series, Petrie (1937) has given a detailed discussion of the factors which determine the rates of import and export of nitrogen by the leaves and so determine their nitrogen content at any time.

These factors, classified in a slightly different manner, are as follows:

(1) the effect of external concentration;
(2) the rate of growth of leaves;
(3) the rate of growth of other parts and the nature of their development;
(4) the capacity of the root system for intake;
(5) internal changes in the leaves leading to a decrease in the net rate of protein synthesis.[4]

These factors will now be considered in relation to the total- and protein-nitrogen data of the present experiments.

(1) The initial external concentration of nitrogen was the same for each treatment, and cannot in itself account for early differences in the rates of uptake of this element by the whole plant or its parts. Fig. 5 shows, however, that long before the end of the growing period the external supply must have been seriously depleted by the developing plant. This is particularly evident with treatments II and III where the absolute content of nitrogen at harvest 5 is slightly in excess of the amount supplied in the culture solutions. With all treatments this depletion of the supply must have contributed towards the decrease in rate of and final cessation of intake with time; and the smaller rate of depletion no doubt accounts for the continuance of intake in I between harvests 5 and 6. The effect of the differential rate of depletion is also reflected in the delayed maximum nitrogen content of the leaves of I and in the very rapid fall in the relative nitrogen content of the leaves of II and III. These effects, however, are not solely attributable to this cause as will be shown below.

(2) It is known that phosphorus as well as nitrogen promotes meristematic activity, and in the early stages of growth this leads to increased leaf formation. In these experiments the extra phosphorus supplied to III produced no marked effect on leaf weight, and there is little doubt that this was due to limiting nitrogen. The increase in leaf weight between I and II, however, is very considerable and, in consequence, the rate of intake of nitrogen and the amount of protein synthesis is at first much greater in II and III.

(3) The influence of this factor is well exemplified in the present experiments. During adolescence the leaves and roots together comprise 75 p.c. of the plant; later the stems and finally the inflorescences grow very rapidly and, during the course of their development, require large amounts of all essential nutrient elements. It is clear from fig. 5 that, in II and III, the external supply of nitrogen becomes exhausted shortly before the exsertion of the inflorescences. The supplies of nitrogen for the inflorescences are thus derived from the other parts of the plant. In the case of I intake of nitrogen ceases at flowering, although an appreciable amount of nitrogen is still present in the external medium. It is possible

[4] The factors defined under the last three headings in paper 3 are here grouped under one general definition.

that withdrawal of phosphorus from the leaves may have caused protein breakdown at this time, so that the leaves would provide a sufficient and closer source of nitrogen for the developing inflorescence.

A further example of the operation of this factor is provided by the data for the primary shoot in Experiment 1 (fig. 6). The absolute nitrogen content of the

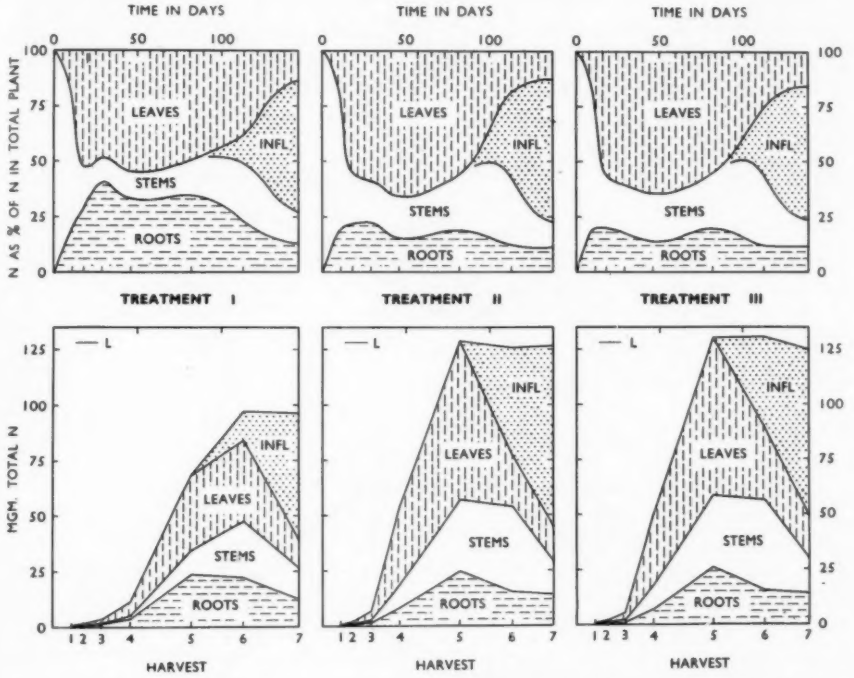

Fig. 5. Graphs illustrating the intake of nitrogen and its distribution within the plant, Experiment 2. Above: the nitrogen present in different parts expressed as percentage of the total nitrogen in the plant. Below: the absolute nitrogen content of the whole plant and its parts. L indicates the magnitude, on the scale of ordinates, of the amount per plant of the nitrogen added to the sand as nutrient solution.

primary shoot of II and III is doubled between harvests 5 and 6, between 6 and 7 there is a substantial loss, and thereafter there are increases up to harvest 9. The period of loss is also a period of rapid growth of tillers, and it is apparent that their nitrogen requirements have set up a temporary state of internal starvation for that element; later the absorption of nitrogen by the roots appears to have kept pace with requirements, and the primary shoot has more than made good its loss. It is probable, however, that some of the nitrogen in the primary shoot is derived

from small vegetative tillers which died early. The curve for I shows the rate of import of nitrogen to have decreased between harvests 7 and 8, suggesting that a similar phenomenon has occurred here. Its later appearance in time is in accordance with the delayed development of tillers, and the slightness of the effect is a further indication of the relatively high nitrogen status of I. The separate curves

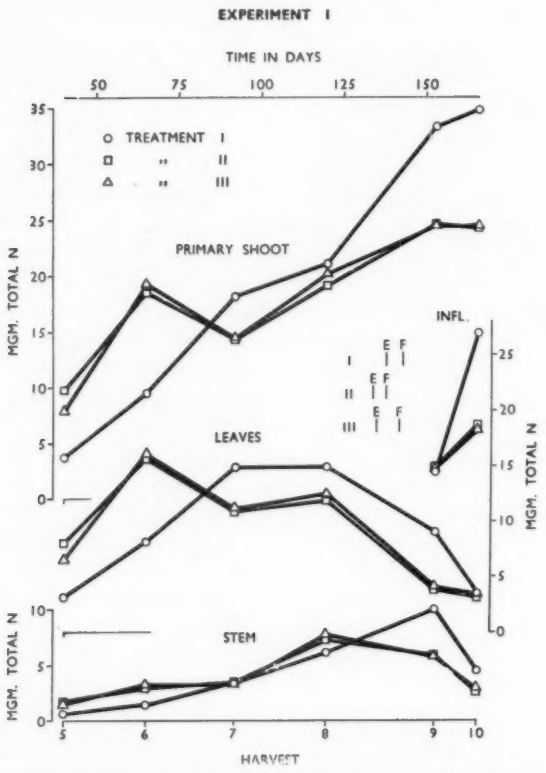

Fig. 6. Absolute amounts of nitrogen in the primary shoot and its parts, Experiment 1. The right hand scale refers both to leaves and to inflorescences.

for the leaves, stems and inflorescences of the primary shoot indicate that the loss of nitrogen with the two higher treatments is sustained entirely by the leaves. Intake by the stems ceases during this period, but the subsequent intake by the whole shoot is almost entirely confined to the stems and inflorescences. In I also, the period of decreased rate of intake of nitrogen by the primary shoot is the time of cessation of intake by the leaves.

Several workers have investigated the interdependence of the tillers of cereals, and Dungan (1931), Smith (1933) and Bartel *et al.* (1935) have shown fairly conclusively that translocation between tillers after the flowering period is possible. The above-demonstrated withdrawal of nitrogen from the primary shoot by the developing tillers shows that transference of mineral elements between tillers may be of normal occurrence under conditions of limited supply. It is probable that the rapid death of non-inflorescence-bearing tillers is due primarily to a withdrawal of nitrogen or phosphorus, and that transference of carbohydrates from these tillers is a secondary phenomenon.

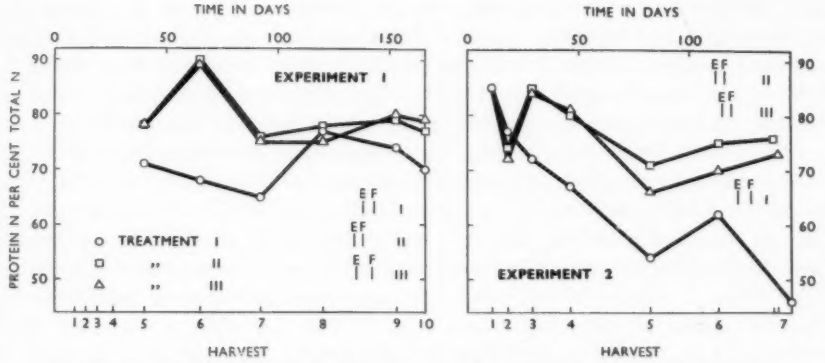

Fig. 7. Protein nitrogen of the leaves expressed as percentage of total nitrogen.

(4) The withdrawal of nitrogen from the primary shoot by the developing tillers probably occurred when the external supply was considerable; this suggests that the root system could not supply nitrogen at a sufficient rate to cope with the requirements of the plant, and this in turn is influenced by the direct effect of external concentration on rate of uptake. The reduction in the root weight ratio of II and III may also tend to limit the capacity of these plants for nutrient absorption in the early stages of growth. That a limitation did occur is suggested also by the loss of nitrogen from the third leaves of II and III after harvest 5 in Experiment 1 (fig. 3), though it is possible that internal metabolic factors may have initiated senescence in these very early leaves.

(5) The factors so far considered influence the rates of protein synthesis and hydrolysis in so far as they determine the concentration of soluble nitrogen compounds in the leaf. The present section will be devoted to other factors, which, acting within the leaf, are additional determinants of these rates.

Eckerson (1931) found a loss of reducase activity and an accumulation of nitrate nitrogen in plants grown without phosphorus; Richards and Templeman (1936), however, found an accumulation of amino- and amide-nitrogen rather

than of nitrate nitrogen in the leaves of phosphorus-deficient plants. In either case it is apparent that protein synthesis is retarded by a limiting supply of phosphorus, and in the present experiments the same factor evidently resulted in an increased content of soluble nitrogen in the leaves of I. This fact provides a partial interpretation of treatment effects on protein nitrogen expressed as a percentage of the total nitrogen (fig. 7); thus at most harvests the values for I are considerably below those for II and III. The internal starvation for nitrogen in II and III would tend to increase the rate of net protein hydrolysis in the leaves at the time of inflorescence formation, but in the case of I, as already suggested, protein hydrolysis may have resulted from a withdrawal of phosphorus rather than of nitrogen. If this were the case it would lead to an accumulation of soluble nitrogen in the leaves of I, and it would account for the very low value for protein nitrogen expressed as a percentage of total nitrogen at the final harvest of Experiment 2. A more pronounced fall in this quantity might also have been obtained for I in Experiment 1 if the final harvest had been made as late as in Experiment 2.

In paper 3 Petrie has discussed the possible effect of a reduction in absolute water content upon protein hydrolysis in the leaves. The present data for total leaves and also for the third leaf provide several cases in which the loss of water is actually preceded by a loss either of total or of protein nitrogen, and there are other cases in which the maxima are coincident. In one instance only, Experiment 2, treatment I, is there a suggestion that water loss may have preceded loss of absolute protein nitrogen from the leaves. It is conceivable, nevertheless, that temporary losses of water due to excess transpiration may influence the rate of protein hydrolysis even when evidence is lacking for the priority of water loss.

The possible effect of a declining rate of carbon assimilation in decreasing the amount of substrate for protein synthesis cannot be evaluated from an examination of these data. Where, as in II and III, nitrogen itself is probably limiting, such a factor is not likely to assume importance.

SUMMARY.

This paper extends the account, given in the second paper of the series, of the effects of different initial supplies of phosphorus on oat plants grown in sand culture. Consideration is now given to the effects on the drifting amounts in the leaves of protein nitrogen, soluble nitrogen and water. Total nitrogen data are also considered for other plant parts.

Up to the time of flowering the leaves of the plants with the lowest supply of phosphorus had very low values for absolute contents of protein nitrogen and water, but the reduction in absolute soluble-nitrogen content was much less marked; after flowering treatment effects were slight. The relative contents of protein nitrogen and water with this treatment were at first depressed below those for the higher treatments, but very soon these relationships were reversed. The relative content of soluble nitrogen was from an early stage considerably increased by low phosphorus supply.

The growth of the plants receiving the two higher supplies of phosphorus was probably limited by the supply of nitrogen, and this is reflected in the similarity of their absolute and relative contents of protein nitrogen, soluble nitrogen and water in the leaves. The initial growth depression with the highest phosphorus supply, however, is associated with decreases in both absolute and relative contents of protein nitrogen and water, and in the absolute content of soluble nitrogen.

The maximum absolute water content of the leaves was preceded by the maximum protein-nitrogen content in the plants with the higher phosphorus supplies, but the position may have been slightly reversed with low phosphorus supply.

For one experiment data for total-nitrogen and water content of the first three leaves of the primary shoot are presented. Treatment relations are similar to those described for the total leaves.

The initial depression in the unit leaf rate with high phosphorus supply is probably determined by the reduced content of protein nitrogen rather than of water. The rate of intake of nitrogen by the roots may be reduced by the high external concentration of phosphorus.

A number of factors that determine the rates of import and export of nitrogen by the leaves are presented in the third paper of the series and are here discussed in relation to the data of the present experiments. The withdrawal of nitrogen from the primary shoot by developing tillers is demonstrated for plants receiving the two higher levels of phosphorus supply.

ACKNOWLEDGMENT.

The author once again desires to record his gratitude to Dr. A. H. K. Petrie for advice and for constructive criticism of the manuscript.

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THE SALT AND WATER METABOLISM OF NEPHRECTOMIZED RABBITS

3. INTESTINAL EXCHANGES FOLLOWING THE INJECTION OF WATER OR SODIUM SALTS.

by

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INTRODUCTION.

In a previous paper (O'Connor, 1937 b) it was shown that the injection of sodium salts into nephrectomized rabbits caused a rise in plasma sodium and chloride; during the two hours following the injection the plasma sodium concentration returned towards normal without any corresponding change in chloride or blood volume; and a consideration of these results led to the conclusion that sodium was deposited in a storage area outside the extracellular fluids without a corresponding movement of water. Likewise, the injection of water caused a transference of sodium from some outside source into the extracellular fluids (O'Connor, 1937 a). The magnitude of this change was about 3 milli-equivs. of sodium per kilo, when a dose of 30 c.c. of water per kilo was given; approximately the same amount was removed from the extracellular fluids following the injection of 5 milli-equivs. of sodium per kilo of body weight.

In the course of the work it was suggested that the intestine could constitute this storage area since the intestinal contents in the rabbit are extremely bulky, forming approximately 10 p.c. of the body weight and containing about 8 milli-equivs. of sodium per kilogram of body weight. This suggestion has been disproved by the work to be reported in this paper.

EXPERIMENTAL PROCEDURE.

The plan of the experiments was identical with those already reported (O'Connor, 1937a, b). Both kidneys were removed from rabbits and the animals were allowed eighteen hours to recover; injections of water (30 c.c. per kilo) or sodium salts (sodium chloride, 3·5 and sodium bicarbonate 1·6 milli-equivs. per kilo) were then given intravenously; two hours later the animals were

[1] Hoffmann la Roche Prizeman.

killed by a blow on the head and the contents of the various parts of the intestine collected for analysis. Comparison was made with control animals, submitted to the same procedure except that no injection was given. In this way the intestinal exchanges were studied under conditions identical with those which led to the conclusion of the existence of such a salt storage.

Diet. In order to obtain some standardization of the intestinal contents these rabbits were maintained on a controlled diet for two weeks before the experiment. The diet consisted of freshly cut green lucern, always taken from the same plot and grown under artificial watering conditions; a fresh supply was obtained each day and each rabbit was allowed 40 p.c. of its body weight daily. Further standardization was not attempted as this process gave sufficiently accurate results for the purpose and involved no interference with the conditions used in the previous experiments. The temperature of the animal house was not controlled and it was not possible to obtain rabbits of the same species for the whole work. Young chinchilla rabbits or Belgian hares were used indiscriminately but these factors appeared to have no significant effect on the results.

Collection of Intestinal Contents. After the animal had been stunned, the stomach, small intestine and caecum were removed between ligatures. The stomach and caecum were weighed; and this figure was taken as the mass of the contents, since the weight of intestinal wall introduces only an insignificant error in comparing such sets of estimations. The stomach and caecum were then opened and sufficient of the contents expressed to carry out the analyses. The contents of the small intestine were collected by "milking" the material into one end of the intestine, and then running the fluid direct into the centrifuge tube.

In experiments where it was desired to study the concentration of the fluid which was in contact with the gut wall, the contents of caecum, small intestine or stomach were centrifuged and the supernatant fluid used for analysis. In the case of the stomach about 10 p.c. of the original material formed a turbid supernatant fluid; in the caecum about 40 p.c. of a highly turbid fluid containing much finely suspended organic material resulted; in small intestine the bulk of the material was a clear bile-stained fluid. In other cases, where the actual gut material was analysed, samples of the required size were taken direct from the gut into crucibles for weighing.

METHODS OF ANALYSIS.

Chloride was estimated by the micromethod of Smirk (1927) which was found to give quite satisfactory duplicates for this purpose.

Sodium was estimated by the zinc-uranyl acetate method described by Butler and Tuthill (1931), using the wet nitric acid plus superoxyl method of digestion with samples of approximately 2 grams; blanks and recovery experiments indicated that the error of the method itself was less than 3 p.c., which is insignificant in this work.

Water Content was determined by drying samples of 0·5–1 gm. at 105°C. for 5 hours. Duplicates agreed to within 0·5 p.c. but it is realized that these are not reliable absolute values. Laviettes (1935) has indicated the difficulties of estimating the water content of such materials, but this simple procedure gives results which are adequate for the purpose of this work.

RESULTS.

The experiments are classed into three groups:

Controls—in which no injection was made.

Sodium injection—in which 3·5 milli-equivs. of sodium chloride and 1·6 milli-equivs. of bicarbonate were injected per kilo of body weight.

Water Injection—in which 30 c.c. of distilled water per kilo was injected.

Stomach.

As is always the case in the rabbit the stomach contained a large amount of semi-digested food, despite the preliminary starvation. In all cases the weight of the stomach constituted between 5 and 9 p.c. of the body weight; there was no recognizable difference between the groups in this respect.

The *sodium* content of the fluid obtained by centrifuging the contents of the pyloric end of the stomach was of the order of 0·5 milli-equivs. per 100 c.c. In a few cases it was a little higher (maximum 1·5 m.e. per 100 c.c.) in the animals injected with sodium salts but these differences were never regular or significant. The *chloride* content of this fluid averaged 16·3 m.e. per 100 c.c. (standard deviation 0·3) in controls but rose to 17·4 (standard deviation 0·2) in the sodium injected animals. There was no significant fall following the injection of water—in fact the figures showed an insignificant rise in the chloride content of this fluid.

Small Intestine.

Drainage of the small intestine in all cases yielded about 15 c.c. of a bile-stained fluid, of which usually one-fifth was solid food material which could be separated by centrifuging; the water content of the untreated contents was always 95–98 p.c. There were no regular differences between the three groups of experiments in respect to water content or volume of this material.

TABLE 1.

Sodium and Chloride Concentrations in Intestinal Fluid.

	Sodium.			Chloride.		
	M.e. per 100 c.c.	No. of Observations.	Standard Devn.	M.e. per 100 c.c.	No. of Observations.	Standard Devn.
Controls	12·9	5	0·5	6·4	7	0·3
After Sodium Injection	14·3	5	0·2	8·5	6	0·6
After Water Injection	13·0	5	0·3	6·7	5	0·3

Table 1 shows the *sodium content* of the supernatant fluid in the three groups. It will be seen that the sodium concentration is not significantly altered by the injection of water but that there is a significant rise following the injection of sodium.

The *chloride* content of the intestinal fluid also rose following the injection of sodium; there was no definite alteration in the experiments in which water was injected (Table 1). Actually it is probable that these differences in chloride content are more definite than statistical analysis would suggest, since in each case the chloride content of the intestinal fluid of animals injected with salt and water was higher than that of the control experiments performed on the same day. These results confirm the observations of Ball (1930) on pancreatic juice and of de Beer, Johnston and Wilson (1935) on the contents of intestinal loops following the injection of salt.

Caecum.

In the rabbit the caecum is very large and anatomically appears to have two portions: (1) The head of the caecum, into which the ileum and colon open very close to one another and comprising about half of the organ. (2) From this a long tail tapers off to the appendix. The wall of this part is folded into plications which form a long spiral valve; it is not possible to milk solid material along the tail of the caecum.

The contents of these two portions were strikingly different. The head contained fluid or semi-fluid material which on centrifuging gave about 40 p.c. of a murky supernatant fluid; the tail contained a dry material from which no fluid could be obtained by centrifuging even at high speeds for prolonged periods. In some cases there was a sharp margin between these phases which could be readily recognized by palpation from the outside; but in other cases the one phase merged into the other. Estimation of the water content showed that the most fluid material was 88 p.c. water whereas the dry material of the tail of the caecum contained as little as 70 p.c. water.

TABLE 2.

Weight and Water Content of the Material in the Two Parts of the Caecum.

	Head.		Tail.		
Nature of Experiment.	Wt. gr. per kg.	Water p.c.	Weight gr. per kg.	Water p.c.	
Control	61	82	18	76	Separation Indefinite.
Control	63	88	24	70	
Control	55	—	12	—	Separation Indefinite. Water at mid caecum 80 p.c.
Control	55	—	14	—	No Definite Subdivision. Water at mid caecum 74 p.c.
Control	46	83	34	71	
Na Injection	50	81	10	68	
Na Injection	37	84	24	72	
Water Injection	100	82	28	72	
Water Injection	55	86	33	75	
Water Injection	95	88	—	—	Whole caecum fluid.
Water Injection	58	—	19	72	

In Table 2 an attempt has been made to indicate the size and water content of these two phases; the site of division was selected by palpation and each part of the caecum weighed separately. The table illustrates the variability of the material and shows no difference between the three sets of experiments. The only difference which could be discerned was in the whole weight of the caecum. In all cases the average weight of the caecum in controls was 76 gm. per kilo (Standard Deviation 2), while in the water-injected animals the figure was 99 (Standard Deviation 6), which indicates a significant increase in the water-injected series. The only two figures available for the weight of the caecum following sodium

injection are 61 and 60 gm. per kilo, which suggests that the caecal contents diminish as the result of the injection of sodium salts.

Chloride concentrations have been estimated only in the fluid obtained by centrifuging the contents of the head of the caecum. The results are shown in Table 3, where it is seen that the mean in the water and sodium-injected animals is higher than in the controls. Statistically these differences are not significant; but the figure for each individual rabbit compared with the corresponding control showed a rise of the magnitude of that indicated by the means. Moreover, the differences are similar to those found in stomach and intestinal fluids, so that it is probable that the differences indicated in Table 3 are real.

TABLE 3.

Sodium and Chloride Fluid Obtained by Centrifuging the Contents of the Head of the Caecum.

	Sodium.			Chloride.		
	Mg./100 c.c. M.e. per 100 c.c.	No. of Observations.	Standard Devn.	M.e. per 100 c.c.	No. of Observations.	Standard Devn.
Controls	12·3	6	0·6	1·8	7	0·2
After Na Injection	14·2	3	0·6	3·0	4	0·7
After Water Injection	13·4	5	0·5	2·7	5	0·3

Sodium Concentration. The sodium contents of the fluids obtained by centrifuging the material from the head of the caecum are also shown in Table 3, and it will be seen that the figures suggest a rise in both sodium- and water-injected animals; statistically neither of these is significant. In the case of the sodium-injected animals, the individual readings were always higher than the corresponding controls, and a similar difference was found in the small intestine; and it is probable, therefore, that the indicated change in the sodium-injected animals is real. No such argument can be applied to the results of the injection of water.

When the attempt was made to compare the sodium contents of the whole material from the various parts of the caecum and colon, no regular results were obtained, even when the sodium content was expressed in terms of water. If, however, the sodium content is plotted against the water content as has been done in Fig. 1 a definite relationship appears. Included in the figures are points obtained by the estimation of the water and sodium content of the faeces in the rectum; these are indicated by the solid points. The points fall close to a straight line which is the same for all three groups of experiments (Fig. 2); towards the lower end the line curves, that for water and control experiments in the opposite direction to that for the sodium group. This probably means that, in the control and water groups sodium is re-absorbed from the intestine more completely than is the case in the sodium group; as will be seen in the discussion the amount of sodium involved is small.

DISCUSSION.

The work has demonstrated certain differences in the contents of the intestinal canal as the result of the intravenous injection of water or of sodium salts. The concentration of both sodium and chloride increased throughout the intestinal canal as the result of the injection of sodium salts; the reverse could not be demonstrated—there was no fall in the ionic content of the intestinal material following the injection of water, the figures actually showing a small rise. It was also found

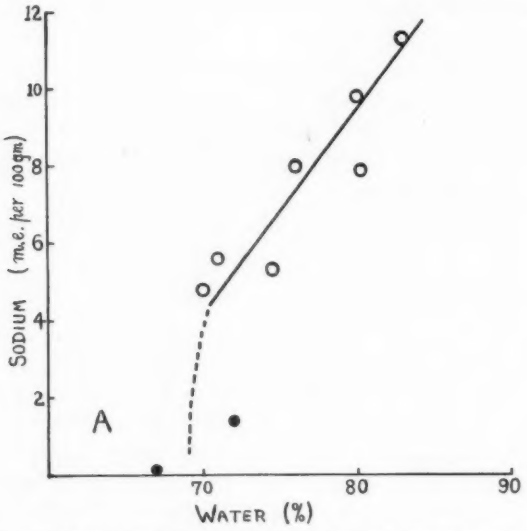

Fig. 1a. Controls. The sodium content of material from caecum or rectum is plotted against the water content.

that the total content of the intestinal canal was increased by as much as 2·5 p.c. of the body weight following the injection of 30 c.c. of water per kilo; it appears probable that there is a loss of weight following the injection of sodium salts. This difference is probably almost entirely due to changes in the water content of the intestine and indicates that the rabbit is an unsatisfactory animal for some aspects of the study of water metabolism; apparently large quantities of fluid may be retained in the intestinal canal of the herbivora. The fluid retained in the intestine was, however, of a composition approximating to that of the blood plasma in respect to its ionic composition, and further analysis is required to consider the relationship of these observed facts to the preceding work demonstrating a sodium storage (O'Connor, 1937 a, b).

It was shown in the previous papers that approximately 3 milli-equivs. of body weight were removed from the extra-cellular fluids as the result of the injection of 5·1 m.e. of sodium salts; likewise a similar amount entered these fluids when water was injected. It is apparent that the observed difference in the sodium content of the material in small intestine is insignificant in this connection; the volume of this fluid was only 15 c.c. per kilo, so that the observed rise of 1·4 m.e. per 100 c.c. in its sodium content is equivalent to a loss from the extra-cellular fluid of only 0·2 m.e. per kilo. Similarly the rabbits only formed about 5 grams of faeces during

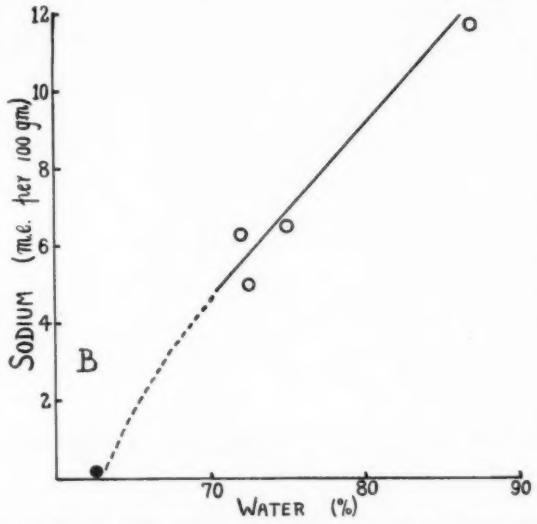

Fig. 1b. Animals injected with water. The sodium content of material from the caecum or rectum is plotted against the water content.

the two hours of the experiment; it was observed that the difference in sodium content of the faeces of the sodium-injected animals was only 4 m.e. per 100 grams; the total loss of sodium in this way is, therefore, only 0·2 m.e. per kilo which is negligible. Moreover in neither of these respects was any difference found between controls and animals injected with water.

Quantitative analysis of the sodium and water exchanges in the caecum is more difficult. Figs. 1 and 2 show that there is no difference between the relationship of the sodium and water content of the caecal material following the injection of water or sodium salts. The caecal contents amount to about 100 c.c. per kilo, and therefore a difference of about 3 m.e. per 100 c.c. in the sodium concentrates would be required if changes in the caecum were to account for the salt storage

previously described (O'Connor, 1937 a, b); this would have been readily recognized in these experiments. Actually there was less than 2 m.e. per 100 c.c. difference between the groups where water and sodium salts were injected.

The slope of the graph correlating the water and sodium content of the caecal material is such as to suggest that an hypertonic solution is being absorbed, if it be assumed that the solid content of the caecum remains unchanged. If, however,

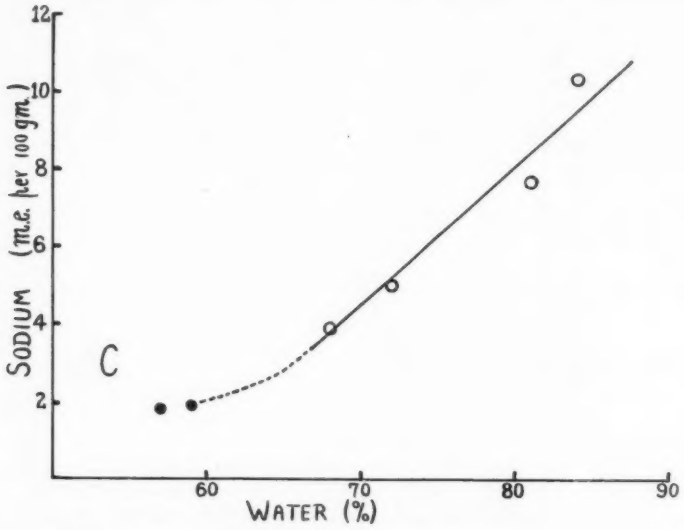

Fig. 1c. Animals injected with sodium salts. The sodium content of material from the caecum or rectum is plotted against the water content.

it is assumed that of 10 gm. of solid material entering the caecum 6 gm. are discharged and 10 gm. of water are produced as the result of bacterial action on cellulose, then curve X of Fig. 32 would be obtained, if the fluid which is reabsorbed has the same sodium content as blood plasma. It is seen that it lies very close to those actually found, demonstrating that there is no necessity to suppose that the fluid reabsorbed by the caecum is hypertonic. There can, therefore, be no grounds for suggesting that the rate of reabsorption could affect the salt content of the body fluids; in any case it was not possible to demonstrate any increase in the dry material of the caecum in the water-injected animals, or decrease in the sodium group, which would suggest that the rate of reabsorption of an an-isotonic fluid formed an explanation of the gain or loss of sodium which occurred in the earlier experiments.

Therefore it is concluded that the extra-renal regulation of the sodium concentration of the body fluids, which previous work has shown to occur in nephrectomized rabbits, is not effected by exchanges of sodium or water with the contents of the intestinal canal.

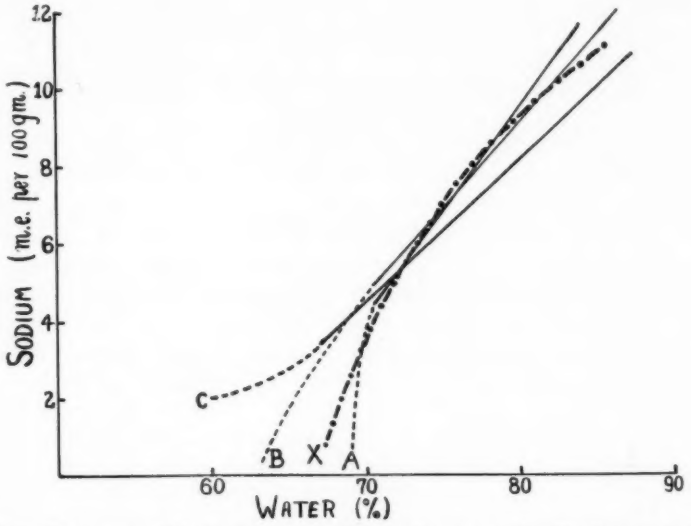

Fig. 2. The curves of Fig. 1, a, b, c are plotted on the same scale. The line marked X is one calculated according to certain assumptions, q.v.

SUMMARY.

1. The sodium, chloride and water contents of the intestinal canal of the nephrectomized rabbit have been examined and the changes, which result from the injection of sodium salts or water, observed.

2. Small changes were observed in the injected animals but these were not of such magnitude to account for the changes of plasma sodium and chloride concentrations observed in previous work.

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THE EFFECT OF THE INJECTION OF WATER OR SODIUM SALTS INTO NEPHRECTOMIZED RATS

by

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INTRODUCTION.

Studies of the distribution of injected water or sodium salts in the nephrectomized rabbit have been previously reported (O'Connor, 1937 a, b). When 3·5 milli-equivs. of sodium chloride and 1·6 m.e. of sodium bicarbonate per kilogram of body weight were injected, there was an immediate rise in plasma sodium of 1–2 m.e. per 100 c.c.; but in the next two hours the value fell again towards normal, being only 0–0·5 m.e. per 100 c.c. above normal two hours after the injection. The chloride showed no corresponding changes; and the conclusion was reached that sodium ions were being removed from the extra-cellular fluids without corresponding shifts of chloride or water (O'Connor, 1937 b).

Conversely, the nephrectomized rabbit was able to restore to normal the low plasma sodium value which resulted from the injection of distilled water; and the conclusion was reached that in this instance sodium ions were entering the plasma, interstitial fluid and lymph (extra-cellular fluids) from some depôt (O'Connor, 1937 a). Further, it has been shown that exchanges with the intestine do not constitute the explanation of these observations (O'Connor, 1938).

These conclusions are contrary to the accepted views on the extra-renal factors in salt and water metabolism; Peters (1935), reviewing the subject, found that physico-chemical forces alone had been demonstrated and was unable to find any evidence either of extra-renal regulatory processes or of a salt storage. There are grounds for believing that salt metabolism of the herbivorous animals is in some respects peculiar, and it was therefore considered desirable to repeat this work on another animal. Dogs unfortunately were not available, and consequently a modified technique was adopted using rats.

EXPERIMENTAL PROCEDURE.

The rats were all adult males of about 200 grams weight. Both kidneys were removed through a dorsal incision under ether anaesthesia and the animals allowed to recover overnight; next

[1] Hoffmann la Roche Prizeman.

morning the test substance was injected subcutaneously into some animals, the others being kept as controls. Two hours after the injection the animals were stunned, the thorax opened and blood collected from the heart. The blood was allowed to clot exposed to air and the serum was used for analysis for sodium by the micromethod of Salit (1932) and for chloride by the method of Smith (1927). The control animals were treated in exactly the same way except that no injection was made.

Dietary conditions. Rats in this laboratory are fed on a mixed diet of meat, milk and cereal with the addition of 0·25 p.c. of common salt. The rats are given their daily ration in the morning and eat it within an hour; the water bottles, however, are always kept full. For these experiments the rats were fed on the day before the operation, and were left in their cage with free access to water until a few hours before the operation, when the water was removed; after operation no food or water was allowed. The temperature of the room was uncontrolled but varied between 20 and 30°C.

The procedure is summarized in the following time schedule:

0–1 hour	Eating food.
1–24 hours	No food but free access to water.
24–29 hours	No food or water.
29 hours	*Nephrectomy.*
29–48 hours	No food or water.
48 hours	*Injection of test fluid.*
50 hours	*Killed and blood collected.*

Objection may be raised to the handling of the blood in contact with air. Van Slyke, Hastings, Murray and Sendroy (1925) have shown that the absolute error in serum chloride or sodium introduced in this way is only 3 p.c., and since all specimens were submitted to the same procedure, it may be assumed that an error of only about 1 p.c. is produced when the estimations are used for comparative purposes only, as is done in this work.

TABLE 1.

Sodium and Chloride Concentrations in Serum following the Injection of Sodium Salts. The Figures in the Last Two Columns are Calculated as in the Example in the Text.

	Sodium. M.e. per 100 c.c.		Chloride. M.e. per 100 c.c.			Increase in extra-cellular fluid, c.c.
Date.	Control.	Injected.	Control.	Injected.	Loss of Sodium.	
2.10.37	14·2	14·9				
	13·8	14·6				
	13·6	14·1				
	13·2					
	Av. 13·7	14·5				
9.10.37	14·2	14·4	9·9	10·9		
	13·8	14·9	10·0	10·3		
	13·8	14·4	9·2	10·7		
		14·6		10·9		
	Av. 13·9	14·6	9·7	10·6	2·1	12
11.10.37	14·0	14·2	9·7	10·7		
	14·2		10·1	10·4	3·6	11
Average	13·9 (±·1)	14·5 (±·1)	9·8 (±·1)	10·6 (±·1)	2·1	15

RESULTS.

Controls.

From Tables 1 and 2 it is seen that consistent results were obtained in the controls, the standard deviation of serum chloride and sodium being only 0·1 m.e. per 100 c.c. in each group. There is, however, a difference between the two sets of controls, the chloride figure being lower and the sodium higher in the second set of experiments; the difference in the chloride figures is statistically significant but that of the sodium figures is not. These differences may be associated with the change of climatic conditions during the month which separated the two sets of experiments; during this time the weather changed from cool to hot and the temperature of the animal house rose from about 25° C. to 30° C. This point is one worthy of further investigation, but at present it is proposed to compare each injected group with the controls performed at the same time and under identical conditions.

TABLE 2.

Sodium and Chloride Concentrations in Serum following the Injection of Water. The Figures in the Last Two Columns are Calculated as in the Text.

	Sodium. M.e. per 100 c.c.		Chloride. M.e. per 100 c.c.			
Date.	Control.	Injected.	Control.	Injected.	Gain of Sodium.	Increase in extra-cellular fluid.
18.11.37	14·0	12·8	9·0	8·0		
	13·8	13·1	9·1	8·5		
	14·6	12·8		8·7		
	Av. 14·1	12·9	9·0	8·3	−0·2	25
23.11.37	13·9	13·5	9·6	9·0		
	12·9	13·9	9·0	8·5		
	14·3	13·9	9·3	8·3		
		13·8				
	Av. 13·7	13·8	9·3	8·6	+3·5	24
1.12.37	14·6	13·7	8·8	8·4		
	14·1	13·7	8·9	8·4		
	Av. 14·3	13·7	8·9	8·4	0	14
5.12.37	14·2	14·9	9·0	8·4		
	14·5	14·1	9·5	8·3		
		13·5				
	Av. 14·3	14·2	9·2	8·3	+4·3	32
7.12.37	15·0	14·4	9·3	8·9		
	14·0	14·2	9·1	8·7		
	14·4	14·3	9·3	8·8		
		13·7		8·9		
	Av. 14·5	14·1	9·2	8·8	+0·5	13
Average	14·2 (±·1)	13·8 (±·2)	9·2 (±·1)	8·5 (±·2)	+2·2	25

The Injection of Sodium Salts.

4 m.e. of sodium chloride and 2 m.e. of sodium bicarbonate were injected per 1,000 grams of body weight; this dose was dissolved in 5 c.c. of water and injected

subcutaneously into one of the hind limbs. Absorption was satisfactory as it was impossible to recognize the site of injection at post-mortem. A comparison of the serum sodium and serum chloride of these rats with the corresponding controls is made in Table 1; it is seen that the sodium concentration rises 0·6 m.e. per 100 c.c. and the chloride 0·8. This confirms the observation made on rabbits that the chloride does not show the same tendency to return to normal as sodium (O'Connor, 1937 b); although more sodium than chloride was injected the final figures show a smaller rise in serum sodium concentration. This point will be analysed further in the discussion.

The Injection of Water.

40 c.c. of water per kilo of body weight was injected into the subcutaneous tissue of the flanks; at first this caused a considerable bulge which disappeared within 30 minutes, and at post-mortem there was no excess of fluid at the site of the injection. As in the corresponding experiments on rabbits (O'Connor, 1937 a) haemolysis was regularly observed as the result of the injection of water, but was only just sufficient to produce a recognizable red colour in the serum.

The comparison of the serum sodium and chloride with those of the controls is made in Table 2, and it will be seen that whereas the average fall of the chloride concentration is 7·5 p.c. that of the sodium is only 3 p.c. Again there appears to be a greater tendency for a restoration of the sodium concentration than of chloride.

DISCUSSION.

In the study of the physico-chemical forces involved in the distribution of salt and water within the body two main principles have been established:

(1) Blood plasma, lymph and interstitial fluid are separated by endothelial membranes which are readily permeable to crystalloids. Consequently these fluids may be regarded as having ionic concentrations which, although not identical, are dependent on fixed factors such as those involved in the Donnan relationships; moreover these fluids will always undergo the same changes. From this standpoint, therefore, these three fluids may be grouped as extra-cellular fluid and their composition be regarded as following that of blood plasma.

(2) The tissue cells contain no chlorine and are impermeable to this ion; consequently all chloride is in the extra-cellular fluid. If this is accepted, it follows that there is very little sodium in tissue cells. Podolsky and Malorney (1935), Fenn (1936), Hastings and Eichelberger (1937) and Harrison, Darrow and Yannett (1936) have all found that the muscle of dogs contains only 0–10 m.e. of intracellular sodium per 1,000 grams of fresh tissue. Harrison, Darrow and Yannet (1936) found no such excess sodium in any other tissue or in the muscle of rabbits or monkeys, but this finding was not confirmed by Podolsky and Malorney who

found excess sodium in the muscle and liver of all animals. In any case the concentration of sodium in cells is less than one-tenth that of potassium. On this basis it is therefore suggested that the cell surface does not allow sodium or chloride to pass, and so the osmotic relationships are dependent on the ionic concentrations of the extra-cellular fluids. When the ionic concentration in these fluids rises, water passes from the intra-cellular to extra-cellular phase; if the ionic concentrations of the extra-cellular phase fall, water passes into the cells. These changes have been demonstrated to occur by Darrow and Yannet (1935) and Hastings and Eichelberger (1937).

TABLE 3.

Calculated Changes in Extra-cellular Volume and Total Extra-cellular Chloride in the Experiments on Rabbits (O'Connor, 1937 a, b). The Figures are calculated as explained in the Text.

Nature of Experiment.	No.		Change in Extra-cellular volume. c.c. per kg.	Change in Extra-cellular sodium. m.e. per kg.
Controls No injection	36		+ 4	+ 1·1
			− 3	− 1·1
	46		− 4	+ 0·4
			− 19	− 2·0
	51		− 15	+ 1·1
			− 17	+ 2·0
	67		− 6	− 1·7
			− 6	− 3·0
			− 6	− 3·0
	68		− 8	− 1·1
			− 8	− 0·8
	69		− 3	+ 0·7
			− 5	− 0·7
		Average	− 7	− 0·4
Injection of 3·5 m.e. NaCl and 1·6 m.e. $NaHCO_3$ per kilo.	58		+ 7	− 4·2
	59		+ 2	− 3·4
	60		+ 14	− 1·3
		Average	+ 7	− 3·0
Injection of 300 c.c. water per kilo.	37		+ 24	+ 1·9
	38		+ 9	+ 1·9
	39		+ 3	+ 4·3
	49		+ 7	+ 2·3
		Average	+ 11	+ 2·6

The results of these experiments may be analysed according to these principles. We accept that the composition of extra-cellular fluid is that of plasma, and that chloride is entirely extra-cellular. We then make the following assumptions:

(1) No chloride enters or leaves the extra-cellular fluids as the result of these injections.

(2) The extra-cellular volume of rats is 300 c.c. per kilo.

Taking the results of the injection of sodium salts as an example, the extra-cellular chloride of the uninjected animals is therefore $\frac{9 \cdot 8 \times 300}{100} = 29 \cdot 4$ m.e. per kilo. But 4 m.e. of chloride were injected per kilo, so that the total extra-cellular chloride two hours after the injection would be 33·4 m.e. per kilo. Since the concentration of chloride in the extra-cellular fluid at this time was 10·6 m.e. per 100 c.c. the volume of the extra-cellular fluid two hours after the injection was $\frac{33 \cdot 4}{10 \cdot 6} \times 100 = 315$ c.c. per kilo.

The sodium exchange may be also calculated. Before injection the extra-cellular sodium was $\frac{300 \times 13 \cdot 9}{100} = 41 \cdot 7$ m.e. per kilo. After the injection it was $\frac{315 \times 14 \cdot 5}{100} \times 45 \cdot 6$ m.e. per kilo. But 6 m.e. were injected so that 2·1 m.e. of sodium per kilo have been removed from the extra-cellular fluids. In Tables 1 and 2 are shown the results of applying this method of calculation to experiments of both series of rats; while in Table 3 are shown the results of applying the same method of calculation to the experiments previously reported on rabbits. The results agree well with these experiments on rats, and in addition it is found that the controls show no significant shift of sodium.

To summarize, the changes which result from the injection of sodium salts are of two types:

(1) As a result of the high sodium and chloride concentration in the extra-cellular fluids the osmotic pressure of these fluids rises and there is a transference of water from the intra-cellular to the extra-cellular phase. This is in accordance with the simple physico-chemical principles outlined by Peters (1935) and agrees with the direct observations of Darrow and Yannet (1935) and Hastings and Eichelberger (1937) on the extra-cellular and intra-cellular phases following large disturbances of the ionic concentrations.

(2) There is a passage of sodium ions out of the extra-cellular fluid, and in this transference there is no corresponding movement of water or the chloride ion. The quantity of sodium involved is about 3 m.e. per kilo in both rabbits and rats. Neither Darrow and Yannet (1936) nor Hastings and Eichelberger (1937) observed this sodium shift; but in both cases large injections were used, and in consequence these small shifts become relatively insignificant and the methods incapable of recognizing them.

In the case of water injections exactly the reverse changes were observed:

(1) As the result of low osmotic pressure of the extra-cellular fluids there is a passage of water into the intra-cellular phase, so that only about 60 p.c. of the injected fluid remains as extra-cellular fluid.

(2) Sodium ions are added to the extra-cellular fluid from some outside source, and again this occurs without shift of chloride or water.

The physico-chemical hypothesis must therefore be modified by supposing that some of the "excess sodium" (i.e. sodium not in extra-cellular fluids) is in a form which is available to the animal and able to appear in the extra-cellular fluid when the sodium content falls. Although the size of this sodium interchange is small compared to the total sodium content of the body, it is of considerable magnitude when considered in relation to the normal daily output and intake; 2 m.e. per kilo in a 70 kilo human being is 8 grams of sodium chloride, and the daily intake is only about 10 grams. Such a depôt is therefore capable of exerting major effects on the salt and water balance.

There is no evidence indicating the site of this sodium storage. Earlier workers claimed that the chloride content of skin was greatly increased following the ingestion of sodium chloride (Wahlgreen, 1909; Padtberg, 1910; Leva, 1915), but it is probable that changes in the quantity of interstitial fluid included in their samples constitute the true explanation of their results. So far as I am aware there is no published work in which the distribution of ingested sodium ions has been directly followed. Harrison, Darrow and Yannet (1936) found excess sodium in bone, which could be the source of the sodium ions which are involved in this work. Harrison (1937) has shown that there is a constant Ca : Na ratio in bone and suggests that the sodium is in the form of a complex salt and is not available to the animal. This discounts but does not disprove the possibility that the sodium of bone has the same storage relationship to sodium metabolism as has the calcium of bone to calcium metabolism.

Harrison, Darrow and Yannet (1936) found that in the rabbit no tissues contained any sodium which could not be accounted for by the interstitial fluid, this being estimated on the assumption that chloride is extra-cellular. Podolsky and Malorney (1936), however, found excess sodium in the liver and muscle of all mammals; while Hastings and Eichelberger (1937) and Fenn (1936) found excess sodium in the muscles of the dog and the frog respectively; Harrison, Darrow and Yannet also found excess sodium in the muscle of dogs. There is therefore justification for doubting the extreme deduction from Harrison, Darrow and Yannet's work and believing that muscle, if not all tissues, contains about 5 m.e. of intracellular sodium per 1,000 grams of tissue. It is possible therefore that some or all of the tissue cells may be the site of this sodium depôt.

In this and the earlier papers (O'Connor, 1937 a, b) the terms "sodium storage" and "sodium depôt" have been used; however, it is obvious that some explanation of these terms is required. If the regulatory processes are considered as methods of maintaining an optimum concentration of the fluids bathing the cells, the terms are justifiable; they merely indicate that there is this area into which excess sodium may be deposited, and from which sodium may be obtained when the sodium concentration of the extra-cellular fluid falls. The previous paragraph suggests that undue emphasis may have been placed on the constancy

of the cellular environment as the manner of body regulatory processes. It is possible that the cells themselves may react to maintain their optimum volume: when the osmotic pressure of the extra-cellular fluid is low, the cell responds by passing sodium ions from the intra-cellular to the extra-cellular phase; the result is a rise in the external osmotic pressure and a fall in the internal osmotic pressure, both of which assist to minimize the changes in cell volume which would otherwise result. Obviously further investigation of the "excess sodium" under varying conditions is required.

SUMMARY.

1. The concentrations of sodium and chloride in the serum of nephrectomized rats have been observed two hours after the injection of water or sodium salts.

2. The results agree with those previously reported for rabbits. It appears that there are two processes involved in the redistribution of injected water or sodium ions:

(a) A transference of water bewteen the extra-cellular and intra-cellular phases as a result of the changed osmotic pressure of the extra-cellular fluids. This is dependent on purely physico-chemical principles.

(b) Exchanges of sodium ions take place between the extra-cellular fluids and some outside area. This transference occurs without corresponding shifts of water or chloride ions, and tends to maintain the sodium concentration of the extra-cellular fluid constant.

3. The site and nature of this depôt area is discussed.

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A COMPARATIVE STUDY OF METHODS FOR THE DETECTION OF THE COLI-AEROGENES GROUP IN WATER

by

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INTRODUCTION.

Although the presence of coliform organisms has long been used as an index of the degree of pollution of water, opinion still differs as to the best method for their detection and estimation, and the sanitary significance of the various types. To try to introduce some uniformity of procedure, the British Ministry of Health (1936) and the American Public Health Association (1933) have each published standard methods for the bacteriological analysis of water. The two methods are, however, different from one another in several important particulars. For the presumptive test, MacConkey broth is recommended in the publication of the British Ministry of Health, and all tubes showing acid and gas within an incubation period of 48 hours are presumptive positives. On account of the rare occurrence of false positives in this medium with English waters (Bardsley, 1934), confirmation is not considered essential. Differential tests for *Bact. coli*, *Bact. aerogenes*, and "intermediate" types may be applied, if desired, to organisms isolated from the presumptive positives by plating on MacConkey agar.

The American Public Health Association recommend the use of standard lactose broth, and those tubes showing at least 10 p.c. of gas in the Durham tube within an incubation period of 24 hours are presumptive positives, while those showing gas within 48 hours are doubtful positives. As it is recognized that false positives occur in this medium, the presumptive positive containing the smallest quantity of water, and all doubtful positives, should be subjected to the confirmation procedure of plating on eosin methylene blue or Endo agar, and testing colonies for lactose fermentation, morphology and reaction to Gram stain. Differential tests for the various members of the coliform group may also be applied to these organisms, if the confirmation tests are positive; but this procedure is not recommended as standard until further evidence is forthcoming to justify the separation of the members of the group on sanitary considerations. The waters with which we have to deal in Victoria often contain large numbers of coliform organisms and

frequently give false positives. The object of the present investigation was therefore to determine which standard method or modification thereof would be most suitable for our needs. On account of the findings of Bardsley (1934) and Atkinson (1934) we do not consider that differentiation of the coli-group is necessary and therefore a presumptive test from which false positives were excluded would not need any confirmation procedure or differential tests. The elimination of false positives is dependent on the medium used, and many media have been devised with this object. Some of them have therefore been included in our tests.

EXPERIMENTAL PROCEDURES AND RESULTS.

THE MEDIUM FOR THE PRESUMPTIVE TEST.

Experiment 1. The ability of the two standard media already mentioned, the crystal violet broth devised by Salle (1930) and a synthetic medium containing lactose, asparagin and brom-cresol-purple, to exclude false positives was compared in an experiment on a sample of water known to give false positives. Thirty tubes of each medium were inoculated with 10 c.c. of water, thirty with 1 c.c. and thirty with 0·1 c.c. All tubes showing gas within 48 hours incubation were designated "presumptive positives" according to the British definition, and were subjected to a confirmation procedure. There were very few such tubes for the synthetic medium after this period, so the incubation period was extended to 72 hours for this medium. The confirmation procedure followed the American Standard Method except that MacConkey agar was used for plating. The tubes were plated out as soon as possible after gas production. When a tube did not confirm[1] by this procedure, it was felt that the negative result might not necessarily be due to absence of coliform organisms, but to a failure in the plating medium to isolate them in the presence of large numbers of other bacteria. Doubts of this kind have also been expressed by other workers (Butterfield, 1933; Horwood and Heifetz, 1934). For this reason a second stage was introduced into the confirmation procedure, and applied to all presumptive positives which did not confirm by the first stage. This second stage consisted of: (1) picking a mixed portion of the most likely looking growth on the MacConkey plate into a lactose fermentation tube, and incubating this tube for not more than 48 hours; (2) if gas were formed a MacConkey plate was stroked, and colonies tested in lactose broth and by Gram stain, as directed by the American standard method. When a tube failed to confirm after this second stage, it was considered to be a false positive. The results for this experiment are recorded in Table 1.

TABLE 1.

Comparison of 4 Media for the Presumptive and Confirmed Tests Using 3 Different Quantities of a Sample of Water.

Quantity of water tested.	No. of tubes inoc.	No. of Tubes Positive.							
		*Lactose Broth.		MacConkey Broth.		Synthetic.		Crystal Violet.	
		Presump.	Confd.	Presump.	Confd.	Presump.	Confd.	Presump.	Confd.
10 c.c.	30	20	12	22	17	20	17	27	18
1 c.c.	30	6	3	3	3	5	5	6	6
0·1 c.c.	30	0	0	1	1	0	0	0	0
Totals	90	26	15	26	21	25	22	33	24

* In this experiment and in all subsequent tests, this medium was modified by the addition of 1 p.c. of Andrade's indicator, to show the presence of acid.

[1] This term is used here and in the succeeding pages to indicate that the confirmation procedure demonstrated the presence of a member of the coli-aerogenes group.

The object of the experiment was to discover a medium giving a few false positives, and reference to Table 1 shows that MacConkey broth and the synthetic medium were, in this respect, the best of the four tried. Lactose broth was the worst, but, as the comparison of the two standard methods was an essential part of the investigation this medium was retained in subsequent experiments. The crystal violet medium, which also gave many false positives, was not included in any further tests.

Experiment 2. The media selected for further study were therefore lactose broth, MacConkey broth and the synthetic medium. Thirty-four samples of untreated water from different sources were tested on these three media, using 5 tubes for each test amount of water (10 c.c., 1 c.c. and 0·1 c.c.) as directed in the British Public Health report. Every presumptive positive was subjected to the double confirmation procedure described for Experiment 1. The results are given in Table 2.

TABLE 2.

Comparison of 3 Media for the Presumptive and Confirmed Tests on 34 Water Samples Using 3 Different Quantities of Each Sample.

Quantity of water tested.	No. of tubes inoc. for each medium.	No. of Tubes Positive. Lactose Broth. Presump.	Confd.	MacConkey Broth. Presump.	Confd.	Synthetic Medium. Presump.	Confd.
10 c.c.	170	151	134	142	121	144	123
1 c.c.	170	109	78	62	43	60	53
0·1 c.c.	170	45	35	12	10	8	7
Totals	510	305	247	216	174	212	183

A comparison of the numbers of presumptive and confirmed tests in Table 2 shows that false positives occur with all three media. If the figures for percentage confirmation are considered, it is evident that there is little to choose between them in this respect, as lactose broth gave 81 p.c. confirmation, MacConkey broth 81 p.c. and synthetic medium 86 p.c.

This was disappointing, particularly in the case of MacConkey broth in which a much higher percentage confirmation was expected as so few false positives are obtained from English waters. Lactose broth, however, had a big advantage over the other two media if the numbers of confirmed positives are inspected (Table 2). This superiority of lactose broth over other media in the detection of coliform organisms has been demonstrated by other workers (Horwood and Heifetz, 1934; Butterfield, 1933; Black and Klinger, 1936) who have agreed that it is by far the most sensitive medium. This fact is brought out even more clearly if the results for each water sample are examined separately, when it is found that 27 of the 34 samples gave higher results in lactose broth than in MacConkey broth and 25 gave higher results in lactose broth than in the synthetic medium. Thus the British Standard Method using MacConkey broth without confirmation is not suitable for the examination of the type of water tested here, on account of the inclusion of false positives and the lack of sensitivity of the medium to the coliform group. The synthetic medium suffers from the same disadvantages. Standard lactose

broth was therefore selected as the best of the three media for the presumptive test although the presence of the coliform group must be confirmed in the positive tubes.

Experiment 3. Although lactose broth was chosen as the best medium of those tested, it was not very satisfactory on account of the necessity for confirmation of presumptive positives. The medium suggested by Dominick and Lauter (1929) was devised, not only for the exclusion of false positives, but also for the detection of *Bact. coli* and not *Bact. aerogenes*. Until the sanitary significance of these organisms is settled, the use of such a medium for the presumptive test is unwarranted. It so happens that this medium is of little use for differentiation within the coliform group (Black and Klinger, 1936). False positives, however, seem to occur only rarely, as various authors (Lauter, 1934; Horwood and Heifetz, 1934; McCants, 1931) report a very high percentage of confirmation. At the same time, the D.L. medium, probably on account of its slight selectiveness for only part of the coliform group, gives a smaller number of confirmed positives than standard lactose broth (Butterfield, 1933; Horwood and Heifetz, 1934). This disability could be overlooked if the D.L. medium provided a presumptive test which did not require confirmation. A comparative test was therefore made with standard lactose broth and D.L. medium on 55 samples of untreated water from various sources, following the procedure described for Experiment 2. The results are set out in Table 3.

TABLE 3.

Comparison of Standard Lactose Broth and Dominick and Lauter Medium for the Presumptive and Confirmed Tests on 55 Samples of Water.

Quantity of water tested.	No. of tubes inoculated for each medium.	No. of Tubes Positive.			
		Lactose Broth.		D.L. Medium.	
		Presumpt.	Confd.	Presumpt.	Confd.
10 c.c.	275	243	173	242	155
1 c.c.	275	122	62	109	53
0·1 c.c.	275	30	22	17	11
Totals	825	395	257	368	219

From a comparison of the numbers in Table 3 for presumptive and confirmed tests for each medium it is obvious that false positives occur quite frequently in D.L. medium. Only 60 p.c. confirmation was obtained with D.L. medium, and 65 p.c. with lactose broth. The total number of confirmed positives was lower for D.L. medium and this agrees with the results already cited from other workers, who found it relatively insensitive to coliform organisms. This defect is not so marked, however, as it was with the media of experiment 2, because only 29 of the 55 water samples tested gave higher results with lactose broth. However, as D.L. medium gave false positives just as readily as lactose broth, no apparent advantage would be gained by its adoption for the presumptive test, and therefore lactose broth must still be retained as the most suitable medium.

THE CONFIRMATORY TEST.

The Type of Positive Requiring Confirmation.

The previous experiments have demonstrated the relatively frequent occurrence of false positives with all media so far tried for the presumptive test. A

confirmatory test must therefore be used, but it is possible that this test need not be applied to every positive tube. The American publication recommends that, in the case of presumptive positives (tubes giving gas within 24 hours), only the tube containing the smallest portion of the sample be subjected to confirmation. This seems to imply that false positives are seldom found among such tubes. The further recommendation that all doubtful positives be subjected to confirmation suggests, however, that tubes showing gas in 48 hours and not in 24 hours are much more liable to give false positives. All the positive tubes obtained during Experiments 1, 2 and 3 except those detected by the synthetic medium were therefore classed as presumptive or doubtful positives according to the American definitions, and all were tested by the confirmation procedure already described. There were 251 presumptive positives, and all confirmed for the coli-aerogenes group, so that no false positives were detected. It must be added, however, that these tubes did not conform simply to the American definition of a presumptive positive, but gave acid as well as 10 p.c. gas in 24 hours. The American definition of a doubtful positive was retained, because, in practice, the presence or absence of acid in these tubes was not found to be related to the presence or absence of the coliform group. There were 968 of these doubtful positives, and only 669 confirmed, so that 31 p.c. were false positives.

It was concluded that no confirmation of presumptive positives was necessary but that all doubtful positives should be subjected to a confirmation procedure for the coli-aerogenes group. This latter point is emphasized by an examination of the collected results for the routine analysis of various metropolitan and country water supplies over a period of two years. Out of 4,433 tubes inoculated, 1,056 doubtful positives were obtained, and of these, only 524 confirmed. Thus, more than 50 p.c. of these doubtful positive tests were false positives. This fact forms a convincing argument for the need of confirmation of all doubtful positives.

The Plating Medium for the Confirmatory Test.

MacConkey agar is recommended by the British Ministry of Health for the isolation of coli-aerogenes organisms in water analysis. The American Public Health Association suggests Endo or E.M.B. agar for this purpose. As MacConkey agar was the medium previously used in this laboratory for routine water examinations, it was adopted for the confirmatory test already described, and used in Experiments 1, 2 and 3. In practice, this medium appeared to be insufficiently selective for the coliform group, and, for this reason, a second stage, which has previously been described, was introduced into the confirmation procedure, giving all unconfirmed tubes a second chance of confirming. The inclusion of this second stage was amply justified by the fact that 126 extra confirmed positives were obtained out of a total of 496. Thus the medium was proved to be unsatisfactory, as 25 p.c. of this total number of confirmed positives would have been recorded as false positives if the second stage of confirmation had not been applied. Even then,

it seemed likely that some true positives could have been missed, and in any case this double confirmation process was much too cumbersome for routine practice.

It was, therefore, desirable to find a more efficient plating medium, which would give as many positives as, if not more than, MacConkey agar, and yet need no second stage of confirmation. Endo, E.M.B., and violet-red-bile-salt agar were selected for trial. The number of positives obtained with each of these media was compared with the number with MacConkey agar, using the double confirmation method with the last mentioned only. Endo and violet-red-bile-salt agar were discarded early in the experiment, as neither gave nearly as many positives as MacConkey agar. E.M.B. agar was found to be very satisfactory, however, and actually gave more confirmed positives than MacConkey agar, despite the advantage which lay with the latter on account of the two stages of confirmation. In actual figures E.M.B. gave 296 positives out of 614 tubes tested, while MacConkey agar gave 277. Moreover, E.M.B. agar gave good, discrete colonies and little growth of non-lactose fermenters. There seems no doubt that E.M.B. is the best of these plating media, and it was therefore adopted in routine practice.

DISCUSSION.

The purpose of this work was to decide upon the most efficient and most rapid method for the bacteriological analysis of Victorian waters. That these differ from the usual type of water tested in the British Isles is shown by the large incidence of false positives in Victorian water, whereas these are rare in the former country. Lactose broth was found to be the most sensitive medium for the coli-aerogenes group in the presumptive test, and, as it did not often exceed other media in the number of false positives, it was considered the most suitable presumptive medium. The results of our experiments with D.L. and MacConkey broth were disappointing in the light of those obtained from other countries, but the failure of these media is no doubt due to the fact that many of our false positives are given by synergic reactions involving two Gram-negative bacilli, and such organisms are not eliminated by either of these media. As a contrast to our findings, the two false positives obtained by Bardsley (1934) in her examination of English waters were due to anaerobes which fermented lactose. The D.L. medium appears, however, to be neither more nor less sensitive to the coli-aerogenes group than MacConkey broth. The finding that "presumptive positives" (amended American definition) do not require confirmation simplifies the procedure to some extent, but the necessity for confirmation of every doubtful positive prevents any appreciable shortening of the coli-aerogenes test.

The superiority of E.M.B. agar over the other plating media tried is evident from the increased number of positives obtained by its use. The reason for this is that it tends to inhibits either the growth or the spreading of non-lactose fermenters.

The use of 5 tubes of each quantity, 10 c.c., 1 c.c. and 0·1 c.c. of the test water is recommended by the British Ministry of Health, and this suggestion was adopted because it was realized that little information can be gained by the use of one tube of the lower quantities in examining Victorian waters in which positive tests frequently occur in these amounts. Halvorsen and Ziegler (1933) have shown that the accuracy of the test increases rapidly with the number of tubes used up to 60, where the deviation is still 40 p.c. above and 35 p.c. below the mean, and that 5 tubes of each quantity give a deviation of 260 p.c. above and 72 p.c. below the mean. However, though this large error is realized, practical considerations limit the number of tubes to five.

SUMMARY.

1. A comparison was made between lactose broth (with indicator), MacConkey broth, crystal violet broth, a synthetic medium, and Dominick and Lauter medium, for use in the presumptive test with Victorian drinking water.

2. The most suitable presumptive medium was found to be lactose broth (with indicator).

3. Eosin methylene blue, MacConkey, Endo and violet red-bile salt agar were compared as plating media for the confirmatory test.

4. Eosin methylene blue agar gave the best results.

5. It was concluded that the confirmation of presumptive positives (those giving acid and gas within 24 hours) is unnecessary and that the confirmation of all doubtful tests is essential.

6. The method adopted for the bacteriological analysis of Victorian drinking water is that given by the American Public Health Association (1933) except that 1 p.c. of Andrade's indicator is added to the lactose broth, and that no confirmatory test is required for presumptive positives.

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THE FALSE POSITIVE REACTION IN THE PRESUMPTIVE TEST FOR BACT. COLI IN WATER

by

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INTRODUCTION.

The frequent occurrence of false positives in the presumptive test for *Bact. coli* in samples of Victorian drinking water was stressed in a previous paper (Atkinson and Wood, 1938). It was shown also that these false positives were only found among doubtful tests as defined by the American Public Health Association (1933). In the present paper the nature of the false positive reaction is discussed together with the relative frequency of the different types and their distribution among the various media tried out for the presumptive test. The criterion for doubtful positives is the formation of gas in lactose broth within an incubation period of 48 hours. It is evident that organisms, other than members of the coli-aerogenes group, are capable of giving this reaction, and Salle (1930) describes false positives from American water samples as being due to three different causes: (1) anaerobes, (2) synergic reactions, (3) sporing aerobes capable of fermenting lactose with the formation of gas. Bardsley (1934), working with English water samples of a very varied nature, found only a few false positives and they were all caused by anaerobes. Of the false positives recorded in our previous paper, 112 were selected at random and submitted to exhaustive tests to establish the cause of the reaction as occurring in Victoria.

EXPERIMENTAL.

Isolation of Cultures.

It was expected that organisms of the same type as those already described by other workers for false positives would be isolated from the tubes examined here. To make provision for the recovery of all these types of bacteria various cultural methods were adopted. Sporing organisms, when present, were obtained by sowing about 0·5 c.c. of the false positive culture into a tube of cooked meat medium, which was incubated for three days at 37°C. A portion of this was then held at 80°C. for 10 minutes, and sown into a second tube of C.M.M. which in turn was incubated at 37°C. From this tube, an agar plate was stroked to obtain sporing aerobes, and a blood agar plate inoculated and incubated anaerobically to isolate anaerobes, colonies of which when present, were picked off into C.M.M.

The synergic type of false positive requires the presence of two organisms, one of which will ferment lactose, giving acid, and the other must be capable of fermenting glucose to give acid and gas, though having no action on lactose. Such synergic pairs may be formed by two Gram negative bacilli or a Gram negative bacillus and a streptococcus. In order to isolate these organisms, a MacConkey plate was sown from the false positive tube, and a pure culture was made from each type of colony appearing on the plate after 48 hours incubation at 37°C. If the cause of the false positive reaction could not be related to the cultures isolated by this procedure, the original tube of C.M.M. sown for the isolation of sporers from the original false positive proved useful because the false positive reaction could be reproduced by sowing therefrom into lactose broth. Also by plating from this C.M.M. tube onto agar and selecting all types of colonies, organisms were frequently obtained which had not been previously detected. Although streptococci sometimes appeared on the MacConkey plates, it was found that whenever smears showed their presence in the original false positive tube they could be isolated from the tube of C.M.M. sown with heated inoculum from the C.M.M. culture already mentioned. All the aerobes obtained by these methods were kept as stock cultures on agar and the anaerobes in C.M.M.

The Determination of the Nature of the False Positive Reaction.

In order to determine the nature of the false positive reaction it was necessary to show that this could be reproduced by the combined growth in lactose broth of some or all of the organisms previously isolated. Great difficulty was experienced at first in reproducing false positives except in the case of those which contained anaerobes. It was found, however, that by combining the organisms in sterile water and inoculating lactose broth tubes with this, the false positives were easily produced. In every case only two different cultures were required to give the reaction.

When a false positive had been reproduced, a portion was plated on MacConkey agar and all types of colony picked off into lactose broth in order to show that the reaction was really a false positive one, and that no coliform organisms were present. When an anaerobe was concerned in the production of a false positive only the associated aerobe grew on the plate and no attempt was made to reisolate the anaerobe; but when bacterial synergism was involved both the organisms comprising the synergic pair were usually recovered. In the latter case, if recombination of these organisms produced the false positive the cause of the reaction was considered to have been established. In the case of the anaerobic type of false positive it was considered sufficient proof of the nature of the reaction if the aerobe isolated from the MacConkey plate could be recombined with the original culture of the anaerobe to give a false positive.

RESULTS.

The Types of Organism Isolated.

Sporing aerobes were found infrequently and in no case did they ferment lactose with gas formation or play any part in the false positive reaction. In this respect they differed from those described by Greer (1928).

All the anaerobes isolated showed colonies which were haemolytic on blood agar. They also gave a stormy clot in milk and were therefore assigned to the *Cl. welchii* group. They did not, however, produce the false positive reaction without the aid of another organism.

Several cultures of streptococci were obtained and all were of the *Str. faecalis* type.

Cultures of Gram negative bacilli were isolated most frequently, and there were two main types, one of which gave the characteristic reactions for the *Proteus*

group. The other type, which fermented glucose, mannite, sucrose and lactose giving acid with or without a small bubble of gas, was assigned to the group referred to under the term *Bact. coli anaerogenes* by Topley and Wilson (1936).

The Types of False Positive.

In doubtful tests which failed to confirm three types of false positive were recognized.

Type 1. False positives due to anaerobes of the *Cl. welchii* group in association with a Gram negative bacillus of the *Proteus* group. This latter organism is required to produce reducing conditions in the medium so that the anaerobe may be enabled to grow and ferment the lactose. In such tubes the indicator is reduced so that acid formation is not made evident. A similar symbiosis may occur with an anaerobe and a member of the coli-aerogenes group, and this would account for the finding of members of this group in some doubtful tests showing gas but no acid.

Type 2. False positives due to a synergic reaction involving two different organisms, one being a non-lactose fermenter giving acid and gas from glucose and the other a lactose fermenter giving acid but no gas. Two such synergic pairs were discovered both of which contained a non-lactose fermenter of the *Proteus* type; but in one case this organism was combined with *Str. faecalis* and in the other with *Bact. coli anaerogenes*. These tubes also show little or no acidity, but here this is not due to reduction of the indicator but to actual lack of acid.

Type 3. False positives which were eventually shown to contain a member of the coli-aerogenes group and have been termed "masked positives". The predominant organism in such tubes belonged to the *Proteus* group and therefore had a spreading growth which possibly is the reason for the difficulty in isolating the coliform organism. The inefficiency of MacConkey agar in this respect has already been discussed by us in the previous paper. It has, however, been suggested by Thompson (1927) that the failure to isolate *Bact. coli* from such tubes is due to the production by that organism of a lethal acidity. Only the weaker strains would be inhibited in this way, and this explanation seems to us to be the more satisfactory as the *Proteus* organism mentioned above did not prevent the isolation of coliform organisms from the great majority of tubes containing both bacteria.

The Frequency of Occurrence of the Different Types of False Positives.

Among the 112 false positives examined there were 84 type 2 (synergic), 24 type 1 (anaerobic) and 4 type 3 (masked positive). Of the 84 synergic false positives only 6 contained streptococci. Therefore, the false positive due to the synergic action of *Bact. coli anaerogenes* and a *Proteus* type occurred most frequently. Anaerobic false positives were also found quite often while masked positives and synergisms containing streptococci were relatively rare. It has been noted by Thompson (1927) and Greer (1928) that the type and number of false

positives varied with the season. This fact was also noticed in the present investigation, for at certain times of the year the synergic action of two Gram negative bacilli was the cause of all the false positives; whereas streptococci and anaerobes were often responsible at another time. Every water sample examined was found capable of giving every type of false positive at some time of the year. The seasonal occurrence of false positives is shown by the fact that in early summer lactose broth showed 81 p.c. confirmation while in winter the percentage confirmation was only 67 p.c.

The false positives tested out during this work were not all obtained from the same presumptive test medium. From a series of tests on one set of water samples (see Experiment 2 in previous paper) we selected 26 false positives in lactose broth, 19 in MacConkey broth and 14 in the synthetic medium, all coming from the same water samples, so that a comparison might be made of the numbers of each type of false positive obtained with each medium. Of the 26 false positives in lactose broth there were 17 synergic false positives of which one contained streptococci; 8 were of the anaerobic type and one was a masked positive. There were 19 false positives in MacConkey broth, and 14 were synergic of which one contained streptococci, 4 were anaerobic and one was a masked positive. There were only 14 false positives examined for the synthetic medium, and 7 were synergic of which one contained streptococci, 5 were anaerobic and 2 were masked positives. It would appear therefore that none of these media exclude any of the different types of false positive. A similar comparison of the types of false positive occurring in lactose broth and Dominick and Lauter medium was made with a series of water samples tested in duplicate in these two media (see Experiment 3 in previous paper). Unfortunately only the synergic type of false positive was evident at the time of these tests and 11 of these false positives were found in lactose broth and 18 in D.L. medium. In no case were streptococci present. We cannot tell from these results whether D.L. medium might not give masked positives or be able to exclude false positives due to anaerobes or streptococci, as none of these reactions were found in lactose broth, and the organisms concerned were presumably absent from the water samples at the season when the tests were made. Further work would be necessary to settle these points. It is certain, however, that D.L. medium will give false positives of the synergic type involving two Gram negative bacilli.

DISCUSSION.

The various combinations of organisms recorded in this paper have been referred to as false positives, and this definition is true in so far as coliform organisms were absent (except in the case of masked positives). If, however, the sanitary significance of the organisms in some of these combinations were taken into account, then many of them could not be considered false positive indications of faecal pollution for *Cl. welchii* or *Str. faecalis* was present in 30 out of 112 false positives

examined. All the rest, except the masked positives, contained *Bact. coli anaerogenes*, the sanitary significance of which is slight, possibly indicating remote pollution. On account of seasonal variation there were times when false positives were due almost entirely to synergic reactions involving this organism. At such a time the coli-aerogenes count would be much too high if such false positives were not excluded by the use of a confirmatory test. It seems therefore that although some false positives are indicative of faecal pollution the greater number, probably all at some times of the year, have no such significance. It is therefore more satisfactory to use a confirmatory test for all doubtful positives and thus remove all false positives. Only four masked positives occurred among the 112 tubes examined, so that the danger of missing true positives on account of the confirmation procedure seems very small and would no doubt be smaller still with the use of eosin methylene blue agar as the plating medium for the confirmatory test, because this medium is much more satisfactory than MacConkey agar for the isolation of coli-aerogenes organisms from doubtful tests.

SUMMARY.

1. The following three types of false positive reactions were found in testing water samples obtained in Victoria:

(a) synergic reactions,
(b) anaerobic types,
(c) masked positives.

2. There were two different synergic pairs both of which contained a member of the *Proteus* group associated in one case with *Str. faecalis* and in the other with *Bact. coli anaerogenes.*

3. The synergic type due to *Bact. coli anaerogenes* and the *Proteus* organism was found most frequently. The anaerobic type also occurred quite often and the anaerobe concerned was always *Cl. welchii.* Masked positives and synergisms involving streptococci were only found rarely.

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